CANADA

Seasonal Variation in Selected Nutrients of a River System

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Water samples were collected biweekly from five locations on the Grand River, Ont., from August 16, 1967, to September 1, 1968. Headwater nutrient levels and associated water chemistry were radically altered by the influence of a small city. Processes of recovery occurred downriver in a primarily agricultural watershed. Levels of dissolved oxygen, carbon dioxide, *p*H, alkalinity, hardness, ammonia, nitrate, and silica followed similar seasonal patterns at all locations. High and widely fluctuating levels of chloride, ammonia, nitrate-N, nitrite, and orthophosphate were evidence of gross enrichment. Nitrate-N, orthophosphate, iron, and sulfate levels varied irregularly during the 13-month period.

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INTRODUCTION

Although the classic investigation in chemical limnology by Ellis (1937) presents certain parameters for the investigation of stream pollution, our knowledge of nutrient changes in a watershed, from source to mouth, is inadequate. Seasonal and short-term changes may differ markedly at various locations in a river system and may have a profound influence on productivity and well-being of stream life.

Among nutrients most commonly accepted as valid criteria of water productivity are nitrogen, phosphorus, silica, sulfate, iron, chloride, and the various carbon forms occurring in the buffer systems of water. In order to document variation of these nutrients at different locations, studies were carried out on the Grand River system, which drains a southern Ontario watershed subjected to various agricultural, industrial, and urban activities.

METHODS

The Grand River watershed is the largest catchment in the Great Lakes basin. Data reported in this paper were gathered at five stations located on the east branch (Speed River) and in the main river below the confluence of the east branch and the mainstream. The area under investigation comprises approximately 3300 km², or about one-half of the total drainage basin.

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Station I is located on the headwater of the Speed River system above the city of Guelph and is bordered by nonagricultural soils described as bottom land or muck. It is surrounded by cedar swamp with only a few small pastures in proximity to the river.

Station II is located on the Speed River 6.5 km below Guelph. This area of the river is not influenced by agricultural activity, but is affected by domestic and industrial effluents from the city of Guelph.

Sampling sites on the lower reaches of the Grand River are station III (immediately below the confluence of the Speed and Grand rivers), station IV (84 km from Lake Erie), and station V (45 km from Lake Erie). The lower portion of the watershed is extensively utilized for intensive mixed agriculture.

Samples were collected biweekly from August 16, 1967, to September 1, 1968, between 10:00 AM and 12:00 noon eastern standard time. All water samples were collected in HCl-washed, and thoroughly rinsed, polyethylene bottles. A single sample at midstream was considered typical of water at station I since comparison of random samples collected indicated that depth and flow promoted an adequate mixing of the water. A composite sample of water (taken from mid-depth at midstream and at 5 m from each bank 15 cm below the surface) was used at the other stations.

All tests for the analysis of water were based on procedures outlined by the American Public Health Association (1962). Analyses were performed in part with a Hach Chemical Company Portable Laboratory, Model DR-EL, standardized regularly to ensure reliable results (Kelso, MS, 1969). No preservative was added to any water sample, but a portable cooler maintained a relatively stable temperature in transit between field stations and the laboratory.

Dissolved oxygen determinations, by the azide modification of the Winkler method, were completed in the field. Free carbon dioxide and total alkalinity determinations were titrimetric; in addition to CO_2 and alkalinity, nitrite was determined in the field by the napthylamine hydrochloride technique modified by the Hach Chemical Company.

Determination of ammonia was by direct nesslerization, and *p*H was measured with a Beckman Zeromatic *p*H meter. Both orthophosphate (inorganic) and polyphosphate (acid soluble) were measured colorimetrically by the stannous chloride method (American Public Health Association, 1962). These properties were determined within 3 hr of collection.

Iron was measured by the 1,10-phenanthroline method (Hach Chemical Company, 1965). Hardness and silica were determined by the ethylenediaminetetraacetate (EDTA) titration and the silicomolybdate method (Hach Chemical Company, 1965).

The mercuric nitrate titration for chloride (American Public Health Association, 1962) was used for all water samples and the brucine method was used for nitrate-N (nitrate expressed as ionic form is 4.41 times NO_3-N). A Hellige turbidimeter was used to determine the amount of suspension derived from the reaction of barium chloride with sulfate. Colour development in tests of ammonia, nitrate-N, and phosphate was followed with a Bausch and Lomb "Spectronic 20."

Discharge measurements for the Grand River system were provided by the Ontario Inland Waters Branch of the Department of Energy, Mines and Resources of Canada.

F values (5%) were calculated by analysis of variance for the randomized complete block design. Significant differences between yearly means for each station were compared by the least significant difference (lsd) test at the 5% level (Steel and Torrie, 1960).

RESULTS

Dissolved oxygen, carbon dioxide, *p*H, alkalinity, and hardness followed similar patterns of seasonal change at all sampling sites and, therefore, the data for station II (Fig. 1) may be considered representative of all stations.

High levels of dissolved oxygen (over 10 mg/liter) occurred at all stations from December 1967 to March 1968. Levels below 5 mg/liter were found at all sampling sites during the summer months; however, the lowest concen-

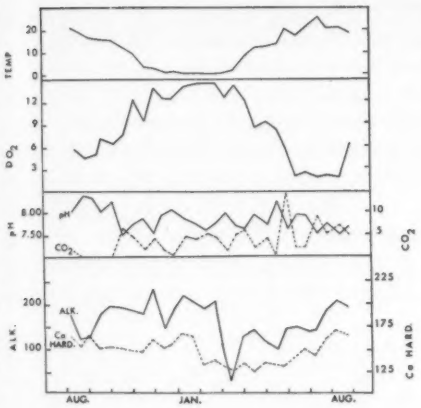

FIG. 1. Changes in temperature (C), dissolved oxygen (DO_2; mg/liter), *p*H, carbon dioxide (CO_2; mg/liter), total alkalinity (alk.; mg/liter), and calcium hardness (Ca hard.; mg/liter), at station II from August 16, 1967, to September 1, 1968.

trations and the greatest range (1.7–15 mg/liter) occurred at station II, below Guelph.

Dissolved oxygen levels followed an inverse relation to water temperature. The maximum temperature (26.3 C) was recorded at stations IV and V on July 17, 1968, and these sites also exhibited the greatest seasonal range (0.1–26.3 C). The least range in temperatures (0.2–16.2 C) occurred at station I.

	Temp (*C*)		Discharge (m^3/sec)	
Station	Range	Mean	Range	Mean
I	0.2–16.2	7.4	0.18–1.43	0.48
II	0.2–26.0	10.9	1.5–27.0	4.8
III	0.2–26.0	11.6	13.1–180.4	40.4
IV	0.1–26.3	11.6	16.3–251.9	57.1
V	0.2–26.3	11.9	17.3–272.0	61.7

Patterns of discharge were similar at each of the five locations. Maximum discharges occurred in March and December, and minimum from June through August. Flow was relatively unstable during January and February.

Free carbon dioxide and *p*H levels followed an inverse relation during the 13-month period (Fig. 1). Carbon dioxide levels varied from 0.0 to 14.5 mg/liter and *p*H from 7.25 to 8.81. Lowest levels of *p*H (7.2–7.5) were accompanied by high levels of carbon dioxide (8.5–14.5 mg/liter) and occurred during periods of high discharge. No consistent period of maxima or minima was found although *p*H was slightly lower, and CO_2 higher, during the winter months.

Similar changes occurred in total alkalinity and calcium hardness; both were consistently highest in late summer, fall, and early winter. Mean levels of alkalinity at the five sampling sites were not significantly different (Table 1). Lower alkalinities (45–50 mg/liter $CaCO_3$) existed during periods of high discharge (Table 2).

TABLE 1. Yearly mean concentrations of selected nutrient characteristics of the Grand River, Ont., measured between August 16, 1967, and September 1, 1968. Any two means joined by the same line are not significantly different by the least significant difference test (5% level).

Characteristic	Station I	II	III	IV	V
Ammonia	0.16	1.47	0.70	0.71	0.65
Alkalinity (total)	Not significantly different (5% level)				
Chloride	16	43	33	32	41
Hardness:					
Total	249	263	247	260	300
Calcium	169	177	165	176	224
Iron	0.04	0.27	0.15	0.19	0.21
Nitrate-N	0.08	0.99	1.05	1.04	1.02
Nitrite	0.014	0.046	0.051	0.039	0.035
Orthophosphate	0.09	1.56	0.84	0.68	0.61
Silica	6.9	6.6	5.3	5.2	5.4
Sulfate	45	65	63	86	246

TABLE 2. Means and ranges (in parentheses) of dissolved oxygen, total alkalinity, iron, chloride, and sulfate located at five stations in the Grand River.

Station	Dissolved oxygen	Alkalinity	Iron	Chloride	Sulfate
I	10.3(4.6–14)	96(50–150)	0.04(0.0–0.13)	16(8–20)	45(24–58)
II	8.7(1.7–14)	96(50–140)	0.27(0.06–0.73)	43(16–87)	65(39–87)
III	9.6(4.7–14)	87(50–125)	0.15(0.0–0.43)	33(15–40)	63(42–135)
IV	9.9(3.4–14)	88(50–130)	0.19(trace–0.38)	32(15–53)	86(44–135)
V	10.9(4.3–14)	83(45–125)	0.21(0.06–0.50)	41(20–64)	246(48–500)

Levels of chloride showed a wide range of concentrations at downstream stations (Table 2).

Of the nitrogen forms examined (ammonia, nitrate-N, and nitrite), levels of ammonia showed similar changes at all sampling sites (Fig. 2) and

concentrations ranged from trace quantities to 2.95 mg/liter. Highest levels occurred during January and February at all stations. However, high levels of ammonia (1.5–2.5 mg/liter) occurred also at station II during the summer months. Station I consistently had the lowest and station II the highest levels of ammonia. Lowest levels of ammonia at all stations (trace–0.22 mg/liter) coincided with high discharges in December and March. Mean seasonal levels of ammonia at the three lowest stations (III, IV, and V) are statistically related (Table 1).

Nitrate-N was extremely variable and showed no obvious cyclic tendency during the year (Fig. 3). High levels (1.24–1.90 mg/liter) appeared irregularly during the winter, and levels usually decreased with increase in discharge.

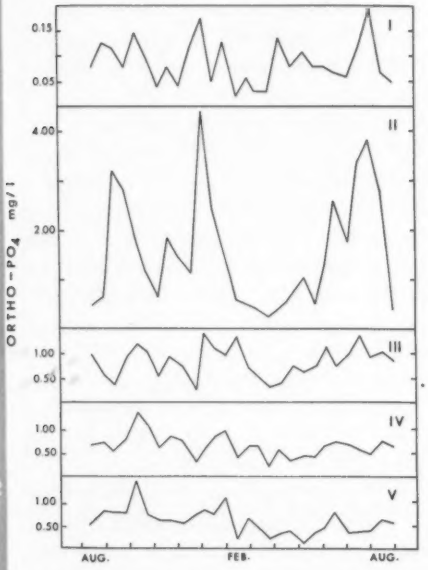

FIG. 2. Seasonal changes in ammonia levels in the Grand River (stations I–V) from August 16, 1967, to September 1, 1968.

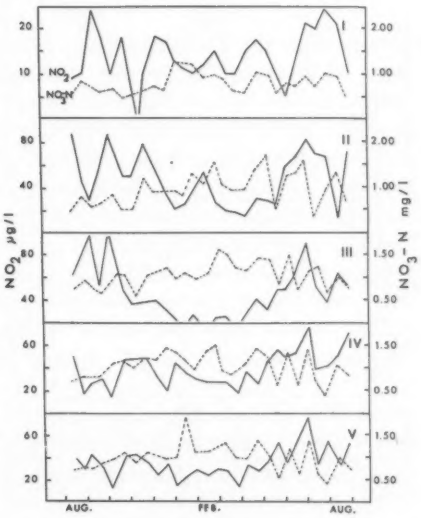

FIG. 3. Seasonal changes in the nitrite and nitrate-N levels in the Grand River (stations I–V) from August 16, 1967, to September 1, 1968.

Levels recorded at station I seemed to be higher during the winter. Mean yearly levels of nitrate-N were not significantly different among stations II–V (Table 1).

Levels of nitrite followed similar changes at all stations, with lowest concentrations generally occurring in winter and spring (Fig. 3). Highest concentrations of nitrite (0.025–0.180 mg/liter) were found during the summer months. Changes in nitrite followed only roughly the changes in nitrate. All of the annual means of nitrite were significantly different at the 5% level (Table 2).

Phosphorus was monitored regularly only as orthophosphate, although polyphosphates were determined for several samples. No regular changes in soluble phosphorus were obvious, although levels seemed somewhat higher during the winter and summer (Fig. 4). Lowest concentrations (0.02–0.21 mg/liter) were found at station I; the highest, and most variable, existed at station II. Polyphosphates were approximately 40% greater than orthophosphates in the eight samples analyzed. Only the mean levels of orthophosphate for stations IV and V were not significantly different (Table 1).

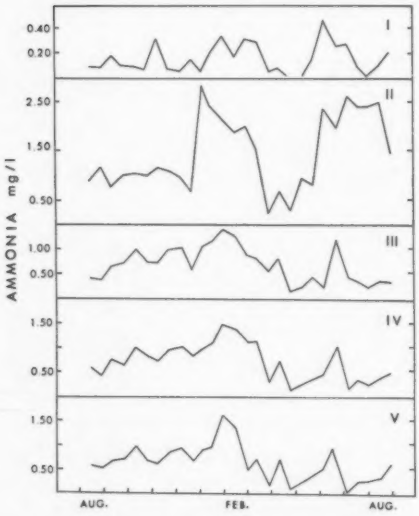

FIG. 4. Seasonal changes in orthophosphate levels in the Grand River (stations I–V) from August 16, 1967, to September 1, 1968.

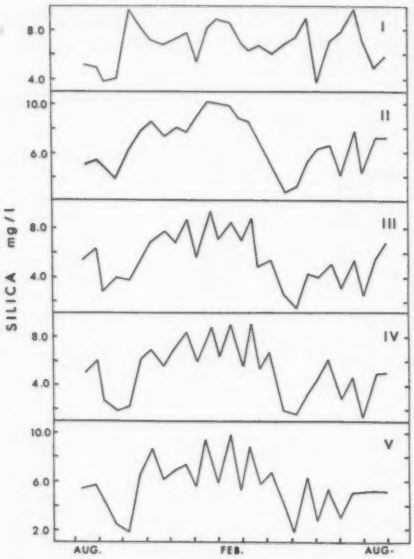

FIG. 5. Seasonal changes in silica in the Grand River (stations I–V) from August 16, 1967, to September 1, 1968.

Highest levels of silica (9–11 mg/liter) consistently occurred during late fall and winter, lowest levels (to 1.1 mg/liter at station III) during spring and summer (Fig. 5). Mean levels of silica at the three downstream sampling sites were not significantly different (Table 1).

Iron concentrations ranged from 0 to 0.73 mg/liter (Table 2) with the greatest range (0.06–0.73 mg/liter) occurring at station II where discharge was the most variable. Levels of iron generally increased with increasing discharge. Mean concentrations of iron at the four downstream locations were not significantly different (Table 2).

Levels of sulfate were extremely variable (Table 2) and generally decreased with increasing discharge. Lowest levels (24–87 mg/liter) were found at stations I and II. A close relation among the yearly means of sulfate existed for stations I–IV (Table 1).

Only ammonia, nitrite, and silica followed consistent regular patterns at all sampling sites; nitrate-N, orthophosphate, iron, and sulfate varied irregularly during the 13-month period.

DISCUSSION

Dissolved oxygen levels, which fell below 5 mg/liter during the summer months at all stations, are evidence of some organic loading of the river water. As samples were taken during midday, the recorded levels may be considered as being near the maximum for each diel period (Kelso and MacCrimmon, 1969). The low levels recorded at station II below Guelph isolate that city as the major source of organic enrichment. The river at that point may be defined as polluted (Ellis, 1937; Doudoroff and Shumway, 1967) with only partial recovery occurring downriver. Seasonal changes in dissolved oxygen levels, because of the effect of water temperature on gas solubility and biological activity, would be expected (Reid, 1961).

Levels of *p*H, carbon dioxide, alkalinity, and hardness in this river system are typical of regions dominated by calcareous bedrock and conform to other levels located in Ontario (Ricker, 1934; Wykes, MS, 1967). The typical inverse relation of carbon dioxide and *p*H was observed, but no abnormal relations were detected at any stations indicating that upsets by acid wastes were not usual. Total alkalinity was highest during late summer, fall, and early winter and lowest during spring and early summer. Hardness followed a similar pattern, since the HCO_3^- and CO_3^{2-} ions contribute to the "calcium hardness" fraction. Forney (1957) also noted this cyclic tendency in farm ponds and attributed it to the utilization of carbonate forms during periods of greatest biological activity in spring and early summer, and the subsequent return of carbonate to solution during reduced activity in response to the lower temperatures of fall and winter.

Comparatively low chloride levels in the headwaters (station I) indicate only modest organic pollution there. Maximum chloride levels (60–87 mg/liter) occurring at station II below Guelph reveals the city effluent as a major source of organic enrichment (American Public Health Association, 1962; Hach Chemical Company, 1965). Although relatively high levels persisted downstream, seasonal variations were irregular. These disturbed levels of chloride, and also hardness, particularly at stations IV and V, reflect the presence of local gypsum deposits composed mainly of $CaSO_4$ with some chloride.

A comparison of annual mean levels of the nitrogen forms (i.e., nitrite, nitrate, and ammonia) indicates a dynamic situation with a wide variation occurring over the watershed. Station II had the highest mean level for all inorganic forms of nitrogen, and the decreasing levels at sites III, IV, and V indicate a progressive "recovery" of the river from organic overloading at Guelph. Concentrations of ammonia above 1 mg/liter indicate organic pollution (Klein, 1962); levels exceeding 2.5 mg/liter may be detrimental or lethal to aquatic organisms (Ellis et al., 1948).

Hutchinson (1957) states that nitrite levels normally follow changes in nitrate and that maximum levels usually occur in winter when the biological demand is least. However, the compatibility of these two nitrogen forms is not distinct in the Grand River where nitrite follows only roughly changes in nitrate levels that are not distinctly seasonal.

Soluble phosphorus in fresh water is believed to exist as orthophosphate, although this organic form represents only a small fraction of the total phosphorus, an essential nutrient for plant and animal life (Reid, 1961). Phosphate levels in the Grand River system undergo large diel variations (Kelso and MacCrimmon, 1969), but seasonal changes are typically irregular rather than cyclic (Fig. 4). The high mean concentration and greatest variability of orthophosphate occurring at station II further indicates that Guelph is a major source of enriching matter. Decreasing levels downstream indicate a partial recovery.

Utilization of silica by certain organisms withdraws this mineral from streams and lakes and, during plankton blooms, the quantity removed may be great (Reid, 1961). The plankton population of the headwater regions of this sytem is low (Wykes, MS, 1967) and would seem to explain the lack of seasonal cycling of silica. Water at the remaining downriver stations is nutritionally richer and subject also to greater seasonal temperature change, which affects the rate of physiological processes. Seasonal variations in silica levels were most pronounced at station II, but all downstream stations exhibited a cycling between summer minima and winter maxima related to variations in plankton production caused by seasonal differences in water temperature.

Since iron can exist in, or in association with, soil or organic particles, and since levels of iron increase with increasing discharge and turbidity, levels of iron (0.0–0.7 mg/liter) reflect the influx of suspended material to the stream.

Sulfate is typically abundant in hard waters but can be introduced by sewage as well as industrial effluents (Hynes, 1966). Levels of sulfate recorded in the Grand River decreased during periods of high discharge resulting from rain or melting snow. The high concentrations of sulfate (over 250 mg/liter) at stations IV and V result almost certainly from local gypsum deposits. In this system, substantial sulfate concentrations (24–599 mg/liter) cannot be considered limiting to algal growth.

The examination of components of the Grand River water generally accepted as nutrients for biological production or criteria for assessing levels of eutrophication illustrates the complexity of the water chemistry within a major watershed serving agricultural, industrial, and urban uses. By monitoring the more-or-less normal water chemistry at an upriver location of the Grand River, it has been possible to define the influence of the nutrient load from a municipal effluent on downstream water quality.

It is obvious that the discharge at Guelph radically upsets "natural" nutrient components. Effects of the addition of organic matter are particularly evident below Guelph in the extreme levels of nitrogen, spectacular changes in phosphate, and the chronic summer depletion of dissolved oxygen.

The progressive decrease in nutrient levels and the effects of eutrophication at downstream sampling sections is evidence of a gradual recovery through natural processes of repurification even though the surrounding watershed is under rather intensive agriculture.

The observed variations in nutrient levels and associated chemical conditions point out the limitation of the common use of spot sampling in the assessment of water quality, particularly if natural nutrient levels and the pattern and range of these fluctuations are not known. Even biweekly sampling, although documenting changes occurring over the river system at specific times of the year, provides limited data for the analysis and interpretation of limnological phenomena. However, it is only through publication of the methodology and findings of long-term investigations, that our limited knowledge of the complex chemistry of river water can be expanded so that increasing problems of eutrophication and water quality control may be understood better and dealt with more effectively.

ACKNOWLEDGMENTS

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The Meromixis of Sunfish Lake, Southern Ontario

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In the meromictic Sunfish Lake the large populations of phytoplankton that are observed periodically were associated with circulation of the mixolimnion. Perhaps some organic dissimilation took place in the lower mixolimnion, recycling nutrients during spring and fall circulation. Phytoplankton was practically absent from the epilimnion during the summer but a population of *Oscillatoria agardhii* remained in the metalimnion, where it was responsible for a pronounced and persistent metalimnetic oxygen maximum. The monimolimnion contained about 5100 mg/liter of dissolved solids and the upper mixolimnion about 2200 mg/liter. Ventilation of the monimolimnion did not always coincide with circulation and subsurface springs may have been responsible. Circulation to the lake bottom was observed only once in 18 months.

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INTRODUCTION

IN A MEROMICTIC LAKE the efficiency of the exchange between the mixolimnion and the monimolimnion to a great extent controls the trophic level of the euphotic zone. Thus in a permanently meromictic lake the monimolimnion acts as a nutrient trap, and, particularly if the importation of nutrients from the watershed is low and the lake is small, sheltered, and relatively deep, primary productivity will be low. Examples of this condition include Krottensee, Austria (Findenegg, 1965), and Green Lake, New York (Jackson and Dence, 1958). In meromictic lakes in which circulation may be complete given favourable meteorological conditions, some return of nutrients to the euphotic zone is accomplished and the primary productivity is consequently higher. An example of this is Hall Lake, Washington (Edmondson, 1963; Edmondson quoted in Frey, MS, 1967). Another consideration, often overlooked, is the recycling of nutrients through circulation in the mixolimnion itself. The effectiveness of this in supplying nutrients to the euphotic zone would in large measure depend on the intensity of organic dissimilation in the lower mixolimnion, the degree of circulation achieved, and its frequency (Findenegg, 1937; Kuznetsov, 1968). This effect apparently would be more applicable to meromictic lakes that have occasional complete circulation. There is, however, little published information on meromictic lakes of this type.

The present study was undertaken to explain the occasional appearance of large algal blooms in the upper waters of Sunfish Lake, a small lake whose phytoplankton productivity is otherwise low. The lake is meromictic, full

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circulation being observed once during the 18 months of study but not subsequently. In the following description several aspects of the meromixis are examined, in particular the influence of the periods of partial or full circulation on the growth of the phytoplankton.

Sunfish Lake is situated at an altitude of 365 m about 8 km to the west of Waterloo in southern Ontario. The lake lies in a small depression surrounded by sandy kames over a Salina (upper Silurian) bedrock of shale, salt, and gypsum (P. F. Karrow, Department of Earth Sciences, University of Waterloo, personal communication). Sunfish Lake and the nearby Spongy and Hofstetter lakes form a series of kettles on the outskirts of the Waterloo Hills. The very small watershed is mostly farmland or mixed deciduous woodland with some hemlock (*Tsuga canadensis*) and cedar (*Thuja occidentalis*) in areas of poor drainage.

The morphometric data of Sunfish Lake is as follows: total area, 8.3 ha; maximum length, 577 m; maximum width, 189 m; maximum depth, 20 m; mean depth, 10.4 m; volume development ratio 1.56; relative depth, 6.15%; maximum depth (m)/$\sqrt{\text{area (ha)}}$, 6.94.

The maximum depth is remarkably deep for the small area of the lake. The relative depth, where the maximum depth is expressed as a percent of the mean diameter (Hutchinson, 1957), and the volume development are high, indicating the pronounced concavity of the lake basin. The main inlet flows only intermittently and a direct passage of water from it to the nearby outlet, bypassing the lake, has been observed on several occasions. There are one or two other very small temporary inflow streams and the presence of several subsurface springs has been reported by local residents. Whether or not these springs are rich in dissolved salts is not known. The littoral is very narrow and mostly free of aquatic plants, but patches of *Chara* sp. occur, the most extensive being around the outlet. The lake bottom is covered by a black sulfide sapropel.

METHODS

Systematic sampling was carried out weekly from February 1966 to July 1967 at a permanent buoy near the deepest point in the lake (Fig. 1).

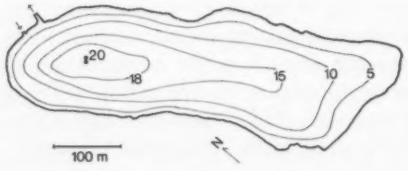

FIG. 1. Hydrographic map of Sunfish Lake showing the position of the sampling station (■). Depths in meters.

Samples of water for chemical analysis and phytoplankton enumeration were collected with a Kemerrer bottle at 1- or 2-m intervals from the surface to the bottom. At each sampling interval the temperature and the percent saturation of oxygen at the observed temperature were measured with an Electronic Instruments Limited Model 15A dissolved oxygen meter. All samples were kept in polyethylene bottles and the further chemical analyses were generally performed

on the same day as collection. *p*H was measured in the laboratory with a Fisher Accumet *p*H meter. Alkalinity (corrected for sulfide), nitrate nitrogen, and silicate (monthly) were measured by the methods in Mackereth (1963). Total phosphate phosphorus and sulfide were measured by the methods of the American Public Health Association (1965). Sulfate was measured by the turbimetric method and iron by the 2,4,6-tripyridal-*S*-triazine method of the Hach Chemical Company (1965). In the comparison of chemical concentrations above and below the chemocline (Table 1) all the analyses except total dissolved solids (TDS) were performed by atomic absorption analysis. The TDS was estimated by evaporation and weighing.

TABLE 1. Some chemical concentrations (mg/liter) above and below the chemocline in Sunfish Lake.

		Depth	
		1 m	18 m
TDS[a]	June 6, 1968	2120	5160
TDS	Dec. 10, 1968	2235	5023
Mn	Oct. 25, 1968	0.01	2.50
Fe	"	Trace	0.03
Zn	"	Trace	0.02
Cu	"	Trace	Trace
Na	"	24.02	34.22
K	"	2.82	2.82
Mg	"	12.17	14.03
Ca	Feb. 10, 1969	55.0	105.0

[a]TDS = total dissolved solids.

The populations of phytoplankton were estimated by sedimentation of 100-ml samples with Lugol's iodine followed by enumeration over an inverted microscope. Units are a single cell, a colony, or a filament 450 μ long (i.e., the diameter of the field of the Whipple eyepiece used).

PHYSICAL AND CHEMICAL CHARACTERISTICS

TEMPERATURE

The isotherms of Sunfish Lake (Fig. 2) are indicative of meromixis. During February and March 1966 the 4 C isotherm was at a maximum of 14 m, the temperature increasing to 5.1 C near the bottom. During the winter of 1966–67 the 4 C isotherm, initially at 13 m in December, gradually rose to about 4 m by the end of February suggesting some process of heating. During the summers of 1966 and 1967 the temperature near the bottom was never less than 5.4 C and on several occasions there was an inverse stratification existing from about 15 m to the bottom. Only during the circulation period in November–December 1966 was there some suggestion of circulation to the bottom.

The thermal profile for November 23, 1966 (Fig. 3), shows a maximum at about 16 m and the profile for July 7, 1967, shows a slight but definite temperature inversion from about 14 m to the bottom.

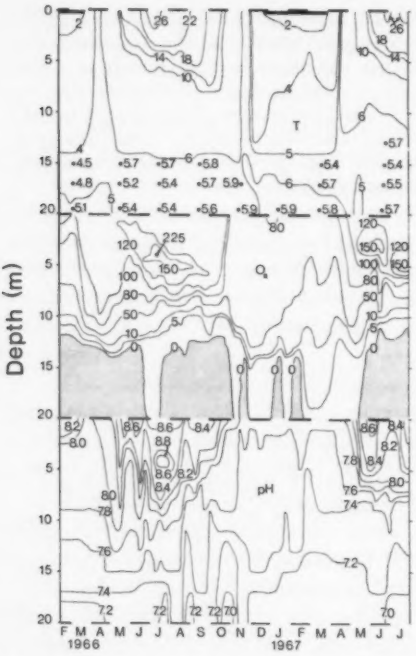

FIG. 2. Isotherms (C) and isopleths of percent saturation of dissolved oxygen and of *p*H in Sunfish Lake, February 1966 to July 1967. Heavy bars are ice cover and stippled areas are anaerobic.

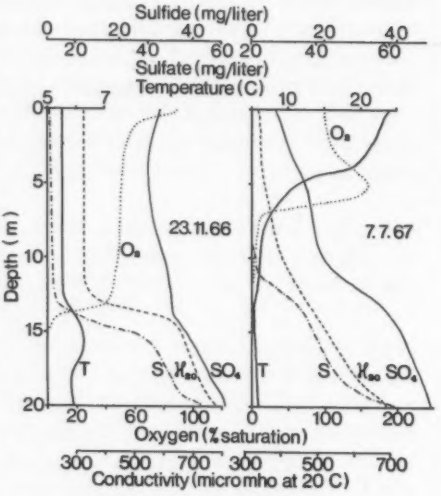

FIG. 3. Profiles of temperature (C), conductivity, dissolved oxygen, sulfide and sulfate on two dates in Sunfish Lake.

DISSOLVED OXYGEN

The isopleths of dissolved oxygen (Fig. 2) clearly indicate prolonged periods of anoxis below 11–12 m and the metalimnetic oxygen maximum during the summers. Although periods of circulation would account for the partial ventilation of the monimolimnion in November–December 1966 and April 1967 no such explanation can account for the oxygen present in July 1966 or under the ice during the winter of 1966–67.

During the prolonged circulation of November–December 1966 the percentage saturation of dissolved oxygen in the mixolimnion fell to less than 80 and, except for one brief interval associated with a brief growth of phytoplankton immediately under the ice, remained less than 80% until circulation the following spring. Very likely this demonstrates the high biochemical oxygen demand of the monimolimnion and lower mixolimnion. On occasions when the dissolved oxygen meter readings were checked against chemical analysis by the standard Winkler method the latter always gave negative results when the meter registered trace (less than 5% saturation) amounts.

Probably, in the presence of H_2S the oxygen electrode is more sensitive than the chemical method.

*p*H AND ALKALINITY

There was a marked decrease in *p*H with depth (Fig. 2). The highest values, 8.0–8.8, were recorded mainly in the epilimnion in the summers of both 1966 and 1967. From November 1966 to May 1967 the *p*H of the surface waters fell to between 7.4 and 7.8. There was little evidence of seasonal changes in the *p*H of the lower monimolimnion, the values being usually around neutrality.

The isopleths of alkalinity (Fig. 4) indicate a marked increase with depth, particularly near the bottom. The concentrations ranged from 75 mg/liter

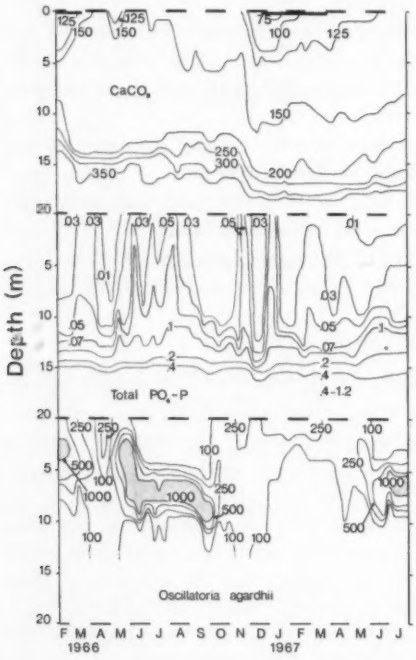

FIG. 4. Isopleths of alkalinity as mg/liter of $CaCO_3$, mg/liter of total phosphate phosphorus, and filaments/milliliter of *Oscillatoria agardhii* in Sunfish Lake, February 1966 to July 1967. Heavy bars are ice cover and stippled areas are 1000 filaments/milliliter or more of *O. agardhii*.

at the surface to over 350 mg/liter at the bottom. A decrease in alkalinity occurred at all depths during the circulation of November–December 1966. There was a partial recovery toward the end of the period of observations.

PHOSPHATE, NITRATE, AND OTHER OBSERVATIONS

There was a pronounced increase with depth of total phosphate phosphorus (Fig. 4) throughout the study period. Concentrations varied from about 0.01 mg/liter near the surface to over 1 mg/liter near the bottom. In general,

the stratification with depth of total phosphate phosphorus in the mixolimnion was most evident during thermal stratification, the isopleths clearly indicating the periods of circulation. Organic dissimilation in the lower mixolimnion in the summer of 1966 is suggested by the general increase in total phosphate phosphorus. The deep circulation of November–December 1966 is clearly reflected in the isopleths yet surprisingly there was apparently little influence on the concentrations below about 15 m.

Amounts of nitrate nitrogen varied inconsistently with depth and time from a trace to 0.25 mg/liter. None was detected below 12 m by the method used. Concentrations of dissolved silicate varied from about 5 to 10 mg/liter.

There was more than twice the concentration of TDS above the chemocline as there was below (Table 1). The most abundant element analysed was calcium. Of the other elements analysed only potassium was more concentrated above than below the chemocline.

On November 23, 1966, the chemocline was evidently between 13 and 15 m (Fig. 3) with the lake circulating to about 15 m. The position of the chemocline was not nearly so evident on July 7, 1967, although a temperature minimum was at 14 m.

Amounts of sulfide near the bottom were usually around 40 mg/liter and sulfate between 60 and 72 mg/liter (Fig. 3). On November 23, 1966, trace amounts of sulfide were found at the surface. Sulfide was generally detectable at 9 or 10 m during thermal stratification of the mixolimnion.

Secchi disc transparency was generally between 4 and 5 m during the summer months but during the periods of circulation it decreased to 2–2½ m.

PHYTOPLANKTON

Over the entire period of observation, by far the most abundant phytoplankter was a species of *Oscillatoria*, tentatively identified as *O. agardhii* Gomont but in some respects resembling the variety *O. agardhii isothrix* Skuja. When observations began in February 1966, a fairly large population existed under the ice and was particularly dense between 2 and 4 m (Fig. 4). During circulation of the mixolimnion in April 1966, the population declined to less than 250 filaments per milliliter. Coincident with the onset of thermal stratification in May the population increased rapidly and by the end of May was in excess of 1000 filaments per milliliter between 2 and 8 m. In June, July, and August the population was practically confined to the metalimnion and densest between 6 and 8 m. In September and early October, the center of the bloom descended further to between 8 and 10 m. With the onset of autumn circulation, the population became scattered fairly uniformly through the mixolimnion. Other phytoplankton were very sparse in the epilimnion during both summers and consisted chiefly of low numbers of several species of Chroococcales. During the winter of 1966–67, there was a small increase in *Oscillatoria* immediately under the ice to about 250 filaments per milliliter but a large population, such as was found in February 1966, did not develop. Also, unlike the previous year, a large population did not develop immediately

after thermal stratification in May 1967. The increase was gradual and not until the 3rd week in June was there a population in excess of 1000 filaments per milliliter between 5.5 and 7.5 m.

Only four other species of phytoplankton formed persistent populations of appreciable abundance. These were *Asterionella formosa* Hass., *Stephanodiscus hantzschii* Grun., *Cryptomonas erosa* E., and *Cryptomonas curvata* E. (Fig. 5). All four species had periods of growth before and during the spring

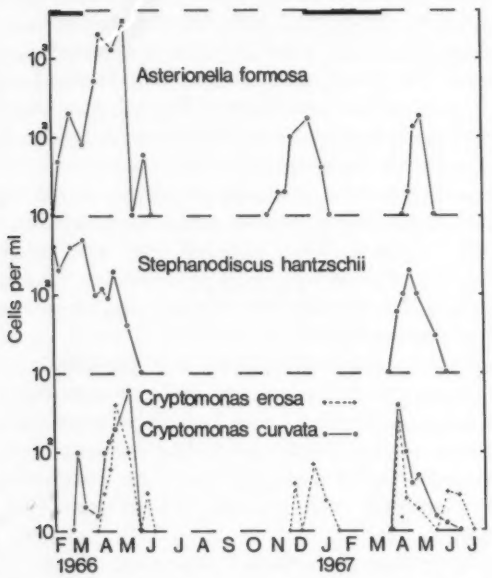

FIG. 5. Variations in populations of four species of phytoplankton in Sunfish Lake, February 1966 to July 1967. Heavy bars are ice cover.

overturn in both years and *A. formosa* and *C. erosa* also had minor blooms in early winter 1966–67. The spring increase in 1966 was greater in each instance than that in 1967, particularly so for *A. formosa*.

DISCUSSION

Of known meromictic lakes in North America, Sunfish Lake is chemically most similar to Green and Round lakes, New York (Likens, MS, 1967). However, Green Lake has high transparency, paucity of phytoplankton, and low productivity (Jackson and Dence, 1958) and in these respects is quite unlike Sunfish Lake. Green Lake is also deeper (50 m), and complete circulation has never been reported. Takahashi et al. (1968) concluded that the cause of permanent stratification of Green Lake is crenogenic, little contribution to the meromixis coming from biological activity within the lake. In Sunfish Lake, since the presence of saline subsurface springs has not been demonstrated, and considering the biological activity within the lake, meromixis is probably largely biogenic, its maintenance assisted by the morphometry

of the lake basin and its sheltered situation. It is highly unlikely that meromixis can be attributed entirely to morphometric features as Northcote and Halsey (1969) postulated for Yellow Lake, B.C. The nature and antiquity of meromixis in Sunfish Lake is currently under investigation by microfossil and chemical analysis of the sediments.

The concentrations of sulfate and sulfide in Sunfish Lake (Fig. 3) are, however, too great to be entirely biogenic (cf. Hutchinson, 1957). In Green Lake the maximum amount of sulfide is about 56 mg/liter (Turano and Rand, MS, 1967) compared with 40 mg/liter in Sunfish Lake. On the other hand the sulfate content of Green Lake is about 1500 mg/liter compared with only 70 mg/liter in Sunfish Lake. The origin of the sulfate in Green Lake has been ascribed to leaching from the surrounding geological formations and possibly the sulfate in Sunfish Lake is derived by leaching from the bedrock, which is known to contain gypsum, or from agricultural runoff.

The decrease in *p*H with depth (Fig. 2) is probably caused by increasing amounts of carbon dioxide, which has a much greater influence on *p*H than the increasing alkalinity (Fig. 4). In Green Lake, Turano and Rand (MS, 1967) point out that the *p*H is more affected by the ratio of carbon dioxide concentration in the monimolimnion to that in the mixolimnion than the corresponding ratio of alkalinity concentrations.

The concentration of phosphate in the monimolimnion of Sunfish Lake is very large and its origin is uncertain. There are no entirely unpolluted waters in the vicinity with which comparisons may be made so the contribution from farming and domestic activities cannot be evaluated. The concentrations of total phosphate in the mixolimnion are dependent partially upon circulation and eddy diffusion across the chemocline and partially upon tropholytic processes in the mixolimnion itself.

The very low amount of soluble iron in Sunfish Lake water (Table 1) is unusual for an inland meromictic lake (cf. Hutchinson, 1957), although ferrous iron is completely absent in the monimolimnion of Lake Mary, Wisconsin, a biogenically meromictic lake (Hutchinson, 1957). In both cases iron is presumably deposited in the sediments as FeS. Conversely, the concentration of TDS (Table 1) is higher than in either Lake Mary or Stewart's Dark Lake and comparable to Round and Green lakes, New York (Likens, MS, 1967). The increase of total dissolved solids in the mixolimnion and the decrease in the monimolimnion between June 6 and December 5, 1968, is probably the result of partial autumn circulation into the monimolimnion.

The occurrence of oxygen in the monimolimnion did not always correspond to periods of deepest circulation. The oxygen may may have entered the lake via subsurface springs, but the irregular nature of the partial ventilation is unexplained. Turano and Rand (MS, 1967) found a concentration of about 1 mg/liter in the monimolimnion of Green Lake. The low concentrations of oxygen in Sunfish Lake were sometimes associated with H_2S. Sorokin (1966) described a similar occurrence in meromictic Lake Belovod and discussed the conditions under which it may occur.

The metalimnetic oxygen maximum (Fig. 2) generally extended from 3 or 4 m to the upper part of the *Oscillatoria* bloom, at about 7 m. In the summer of 1967 the uppermost limit of the oxygen maximum was shallow, averaging 3–6 m. In September 1966 the lower edge of the *Oscillatoria* bloom was in water of less than 10% oxygen saturation and in the presence of at least 1 mg/liter of hydrogen sulfide. In all important respects the behaviour of *O. agardhii* in Sunfish Lake is similar to that described in Myers Lake, Indiana (Eberly, 1959), and McLish Lake, Indiana (Eberly, 1964a), both of which have metalimnetic oxygen maxima. Eberly (1964a) uses the ratio maximum depth (m)/$\sqrt{\text{area (ha)}}$ and suggests that it has to exceed 3 before a metalimnetic oxygen maximum can develop. In Sunfish Lake this ratio is 6.94. Eberly (1964b) reviews the occurrence of metalimnetic oxygen maxima in lakes of the world and their morphometric characteristics. Ninety percent of the lakes had a volume development ratio of greater than 1.00; in Sunfish Lake the ratio is 1.56, indicating the pronounced concavity of the lake basin. Another ratio used by Eberly is relative depth. In Sunfish Lake it is 6.15, compared with 4.16 for McLish Lake and only 2.94 for Myers Lake (west basin). Included in Eberly's review are at least four lakes possessing metalimnetic oxygen maxima that are also meromictic. Their relative depth ratios vary from 1.53 to 10.21 and inversely with their area. Thus Sunfish Lake undoubtedly is related to the class of lakes found in Wisconsin and Indiana that show metalimnetic oxygen maxima or meromixis or both. They are all of glacial origin and have a significantly higher relative depth ratio than lakes in the same area without metalimnetic oxygen maxima or meromixis. Similarly the small size and high relative depth of Sunfish Lake make it comparable to a group of Japanese lakes with metalimnetic oxygen maxima (Eberly, 1964b).

The periods of growth of major species of phytoplankton other than *O. agardhii* in Sunfish Lake (Fig. 5) were associated with circulation of the mixolimnion. In every case the population declined upon, or shortly after, thermal stratification. In spite of the deep circulation and considerable exchange across the chemocline in November–December 1966 the populations in December 1966 and in the following spring were not so great as in the spring of 1966. However, the nature of the autumn 1965 circulation was not studied.

It is improbable that phosphate is limiting at any time, though nitrate might be. The isopleths indicate little association between the amounts of phosphate and the populations of *O. agardhii*. In the epilimnion in summer the concentration of total phosphate phosphorus never fell much below 0.01 mg/liter, yet the total population of phytoplankton from June to September in the upper 3 or 4 m rarely totalled more than 10 cells/ml at any time. Recently Rigler (1968) postulated that the concentration of inorganic phosphate in lake water is much lower than indicated by the molybdenum blue test. If this is so, lack of phosphate may perhaps limit growth in Sunfish Lake. It is equally possible that iron or some other substance is limiting. The meromixis of Sunfish Lake undoubtedly hinders the return of nutrients from the

monimolimnion but this alone cannot explain the low productivity of phytoplankton in the epilimnion during the summer. The same features of morphometry that contribute to the meromixis of Sunfish Lake must also help maintain a very stable epilimnion, allowing little eddy diffusion across the thermocline and thus preventing any return of nutrients except during the spring and fall circulations.

ACKNOWLEDGMENTS

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Effects of Coenzyme A and Carnitine on Fatty Acid Oxidation by Rainbow Trout Mitochondria[1]

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BILINSKI, E., AND R. E. E. JONAS. 1970. Effects of coenzyme A and carnitine on fatty acid oxidation by rainbow trout mitochondria. J. Fish. Res. Bd. Canada 27: 857–864.

The effects of coenzyme A (CoA) and carnitine on the oxidation of 0.1 mM K-palmitate-1-^{14}C and K-oleate-1-^{14}C by mitochondrial fractions from lateral line muscle, white muscle, heart, liver, and kidney was studied in rainbow trout (*Salmo gairdneri*). CoA (0.01–0.10 mM) stimulated the oxidation of fatty acids by mitochondria from lateral line muscle and heart. With all the preparations of mitochondria, fatty acid oxidation was increased by addition of carnitine (1.0 mM) and in the presence of carnitine (1.0 mM), CoA (0.1–1.0 mM) gave a further increase in oxidative activity. Mitochondria from lateral line muscle and heart showed the same range of maximal activity (oxidized substrate at 15 C = 180–200 nmoles/mg N per hr), which was considerably above that found with mitochondria from other tissues. The results suggest similarities in the energy-supplying metabolism in lateral line muscle and heart of trout with respect to the utilization of fatty acids.

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INTRODUCTION

THOUGH THE MITOCHONDRIAL OXIDATION of fatty acids to CO_2, mediated by the enzymes of β-oxidation and tricarboxylic acid cycle, is an energy-yielding metabolic process widely distributed in nature (Stumpf and Barber, 1960), there are indications that its importance varies in different animal tissues (Fritz, 1961; Norum, 1966; Drummond, 1967; Beenakkers et al., 1967; Bass et al., 1969). With reference to fish, it is known that red lateral line muscle has a greater ability than white muscle to oxidize fatty acids (Bilinski, 1963; Jonas and Bilinski, 1964; George and Bokdawala, 1964; Dean, 1969). This directly reflects the difference between the energy-supplying metabolism of these two types of fish muscle, which is also supported by other evidence (for review see Bone, 1966; Bilinski, 1968). The general properties of the fatty acid oxidizing system in lateral line muscle mitochondria were the object of an earlier study (Bilinski and Jonas, 1964). To elucidate further the function of the red lateral line muscle in the energy metabolism of fish, particular attention was focused in the present work on the comparative aspects of the effect of CoA and carnitine on the oxidative activity of mitochondria isolated from various fish tissues.

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[1]The sixth paper in a series on utilization of lipids by fish.

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MATERIALS AND METHODS

Fish

Rainbow trout (*Salmo gairdneri*) obtained from a commercial hatchery were fed with New Age Complete Trout Fingerling Pellets (J. R. Clark Co. Ltd., Salt Lake City, Utah). They were about 2 years old, weighing 200–300 g. The fish used had not been fed for about 20 hr prior to being killed.

^{14}C-Labelled Fatty Acids

Palmitic acid-1-^{14}C and oleic acid-1-^{14}C were obtained from New England Nuclear Corp. They were diluted with the corresponding nonradioactive compounds, converted to potassium salts, and their solutions were adjusted to *p*H 7.3. They had the specific activities of 250,000 counts/min per μmole and 300,000 counts/min per μmole, respectively. Immediately prior to use as substrate, the fatty acid salts were complexed with bovine albumin (fatty acid/albumin molar ration, 7:1).

Preparation of Mitochondrial Fractions

For the isolation of the mitochondrial fractions (DeDuve, 1967) the previously described procedure (Bilinski and Jonas, 1964) was used with only minor modifications. The same technique was employed with the various types of tissue and the starting material was obtained by pooling, in equal weight, the tissue from three fish. Homogenization was accomplished without prior digestion by bacterial proteinase, by gently grinding tissue with sand in fortified 0.4 M sucrose (Azzone and Carafoli, 1960). Centrifugation was conducted at 600 *g* for 10 min and at 11,000 *g* for 15 min. The mitochondrial fractions were washed once with 0.25 M sucrose without additives and after centrifugation they were resuspended in this medium. Aliquots of 0.25 ml of the mitochondrial suspension were employed for incubation and for total nitrogen determination by Kjeldahl method (Steyermark, 1951, p. 134.)

Incubation Procedure

Incubation was conducted at 15 or 25 C in refrigerated model of a Warburg respirometer, using conventional 20-ml vessels with one side arm. The total volume present in the main compartment amounted to 2.0 ml; the side arm contained 0.4 ml 62.5% citric acid, and a circular piece of fluted Whatman No. 1 filter paper (20 mm diam), soaked with 0.1 ml 10% KOH, was present in the center well. The standard incubation medium had the following composition (Bilinski and Jonas, 1964): NaK phosphate buffer, *p*H 7.4, 20 mM; KCl 50 mM; $MgCl_2$, 7.5 mM; ATP, 1.5 mM; α-ketoglutarate, 0.5 mM. The ^{14}C-labelled substrate, complexed with bovine serum albumin, was also placed in the main compartment. The other constituents of the medium were as indicated in the tables. Freshly prepared mitochondrial suspension (approximately 0.5 mg N) was introduced into the main compartment of chilled Warburg vessels. After an initial equilibration period of 5 min, incubation was carried out for 60 min with air as gas phase. At the end of the incubation, citric acid was tipped from the side arm to stop the reaction and liberate CO_2 dissolved in the medium. Shaking was then continued at 25 C for 1 hr.

Determination of Radioactivity

The radioactivity present in the respiratory CO_2 was determined by direct counting of moist KOH filter papers, using the scintillation technique described by Buhler (1962). The scintillator solution used (10 ml) had the following composition: toluene–dioxane–methanol–naphthalene (350:350:210:73, v/v/v/w), 0.46% 2,5-diphenyloxazole (PPO), 0.008% 1,4-bis-2-(5-phenyloxazolyl) benzene (POPOP). The reliability of the radioactivity assay method was confirmed under the experimental conditions used; the recovery of $^{14}CO_2$ showed a linear relation with the amounts of standard solution of $Na_2{}^{14}CO_3$ added to the incubation medium.

RESULTS

The effects of the addition of CoA and carnitine on the oxidation of long chain fatty acids by trout mitochondria were determined at 15 C for

various types of muscular tissue (red lateral line muscle, white muscle, and heart) and for two organs (liver and kidney) (see Tables 1 and 2). The composition of the standard incubation medium was the same as that used in an earlier study, in which cofactors requirement and general properties of the fatty acid oxidizing system were established for mitochondria from lateral line muscle of rainbow trout (Bilinski and Jonas, 1964). The oxidative activity did not show a complete dependence on externally added carnitine or CoA, as preparations from all tissues tested were active without being supplemented by these compounds. Under these conditions, however, only comparatively low levels of activity were observed. The addition of CoA (0.01–0.01 mM) to the incubation medium resulted in a pronounced stimulation of the oxidative activity of lateral line muscle (Table 1) and heart mitochondria (Table 2). Carnitine (1.0 mM) stimulated the oxidation of long chain fatty acids by all types of mitochondria. This stimulation was stronger with lateral line muscle and heart than with other tissues. The addition of CoA to the medium fortified with carnitine resulted in a further pronounced increase of the activity of all the mitochondrial preparations. The maximum level of oxidative activity, reached by the various types of mitochondria, after the stimulation by carnitine and CoA, differed markedly, the mitochondria from lateral line muscle and heart showing considerably higher activity. The results described above were obtained using oleate and palmitate as substrate. Though the same general trend was observed with either of the two acids, in several instances the former tended to be more readily oxidized.

In a more limited series of experiments the effects of carnitine and CoA were determined using a 25 rather than 15 C incubation temperature. The activity at 25 C was generally 5–10 times higher than at 15 C, but available data indicate essentially the same type of response to the additives. The results showing the requirements for ATP (see Table 1) essentially confirm the observation made earlier with fish mitochondria in absence of added carnitine (Bilinski and Jonas, 1964; Brown and Tappel, 1959).

There were pronounced differences in the amounts of mitochondrial fractions isolated from various fish tissues, white muscle showing the lowest yield and liver the highest (Table 3). Since, in the isolation procedure used, no attempt was made to obtain a complete recovery of mitochondria, these data have only a comparative value. The oxidative capacity of various tissues, based on the yield of mitochondria and their activity in presence of carnitine and CoA, indicated the highest values for lateral line muscle and heart, both in the oxidation of palmitate and that of oleate (Table 3).

DISCUSSION

The results showing the similarities between mitochondria from trout heart and lateral line muscle appear of special interest as they provide further evidence that fatty acid oxidation plays a prominent role in the energy metabolism of fish red muscle. It is known from studies with mammals that cardiac muscle is almost entirely dependent upon aerobic metabolism for energy

TABLE 1. Oxidation of fatty acids by mitochondria from lateral line muscle, white muscle, and liver, expressed in nmoles substrate converted to $^{14}CO_2$/mg N per hr. Incubation temperatures, 15 and 25 C; substrate, 0.1 mM.

		15 C		25 C
		K-Oleate-1-^{14}C	K-Palmitate-1-^{14}C	K-Palmitate-1-^{14}C
		Lateral line muscle		
Standard[a]		1.38±0.04[b]	0.53±0.05	6.50±0.10
+CoA	0.01 mM	2.69±0.19	3.46±0.45	–
+CoA	0.10	7.73±0.26	7.58±0.69	32.50±1.93
+Carnitine	1.00	115.30±2.36	53.17±4.49	–
+Carnitine	1.00			
+CoA	0.01	194.30±1.43	182.00±1.70	–
+Carnitine	1.00			
+CoA	0.10	200.30±3.13	180.67±2.66	187.30±8.06
+Carnitine	1.00			
+CoA	0.10			
−ATP	1.50	–	–	70.00±1.13
		White muscle		
Standard		0.03±0.01	Nil	0.13±0.03
+CoA	0.01 mM	0.09±0.01	Nil	–
+CoA	0.10	0.07±0.01	Nil	0.32±0.01
+Carnitine	1.00	1.13±0.10	0.16±0.02	–
+Carnitine	1.00	8.48±0.44	1.59±0.01	–
+CoA	0.01			
+Carnitine	1.00			
+CoA	0.10	9.78±0.64	1.87±0.04	17.17±0.47
+Carnitine	1.00			
+CoA	0.10			
−ATP	1.50	–	–	0.79±0.02
		Liver		
Standard		0.11±0.01	0.50±0.04	6.70±0.10
+CoA	0.01 mM	0.16±0.01	0.23±0.05	–
+CoA	0.10	0.17±0.01	0.30±0.05	3.61±0.16
+Carnitine	1.00	3.23±0.48	0.99±0.10	–
+Carnitine	1.00			
+CoA	0.01	13.83±1.69	4.79±0.46	–
+Carnitine	1.00			
+CoA	0.10	12.60±0.61	8.98±0.51	60.00±3.59
+Carnitine	1.00			
+CoA	0.10			
−ATP	1.50	–	–	2.29±0.09

[a]For the composition of the standard incubation medium see Materials and Methods.
[b]Mean value ± standard error from three incubations.

TABLE 2. Oxidation of fatty acids by mitochondria from kidney and heart, expressed in nmoles substrate converted to $^{14}CO_2$/mg N per hr. Incubation temperature, 15 C; substrate, 0.1 mM.

		Kidney	Heart	
		K-Oleate-1-^{14}C	K-Oleate-1-^{14}C	K-Palmitate-1-^{14}C
Standard[a]		0.46±0.02[b]	0.75±0.08	0.81±0.24
+CoA	0.01 mM	0.77±0.02	3.92±0.55	3.38±0.11
+CoA	0.10	0.61±0.01	4.27±0.32	2.72±0.07
+Carnitine	1.00	4.88±0.58	29.40±1.08	52.10±1.43
+Carnitine	1.00			
+CoA	0.01	31.33±0.30	184.50±9.49	108.17±3.15
+Carnitine	1.00			
+CoA	0.10	40.77±0.85	192.30±12.17	118.83±6.22

[a]For the composition of the standard incubation medium see Materials and Methods.
[b]Mean value ± standard error from three incubations.

TABLE 3. Comparison of the oxidative capacity of various tissues, expressed in nmoles substrate converted to $^{14}CO_2$/g tissue per hr at 15 C.[a]

Tissue	Yield of mitochondria (*mg N/g tissue*)	Oxidative activity	
		K-Oleate-1-^{14}C	K-Palmitate-1-^{14}C
Lateral line muscle	0.63±0.05(6)[b]	126.19	113.82
White muscle	0.16±0.01(4)	1.56	0.30
Liver	2.85±0.36(6)	35.91	25.59
Kidney	1.65	51.69	–
Heart	0.88±0.09(2)	169.22	104.57

[a]Values calculated from the yield of mitochondria and their specific activity in standard incubation medium supplemented with carnitine (1.0 mM) and CoA (0.10 mM) (see Tables 1 and 2).
[b]Mean value ± standard error, and number of determinations in parentheses.

(Masoro, 1968, p. 227) and that fatty acids represent the most important fuel of heart in the postabsorptive state (Bing, 1965). The recent study of Bass et al. (1969), on metabolic differentiation of distinct muscle types in warm-blooded animals shows also that the cardiac muscle and the red skeletal muscle have some common characteristics at the level of enzymic organization.

The exact role of carnitine in mitochondrial metabolism is not yet fully understood (Fritz, 1968; Bremer, 1968), but there is strong evidence that this compound acts by facilitating the transfer of extramitochondrial fatty acids to the site of oxidation in mitochondria (Bremer, 1962; Fritz and Yue, 1963). In the series of enzymic reactions postulated in this process, the extramitochondrial free fatty acids are converted to acyl CoA esters before reacting with carnitine to yield acyl carnitine. In this form they may cross

the mitochondrial barrier, and after being reconverted to acyl CoA they can then be oxidized within the mitocnondria under the action of the enzymes or β-oxidation and the tricarboxylic acid cycle. The stimulation by carnitine of the oxidation of fatty acids by fish mitochondria implies that the two initial reactions (formation of acyl CoA and acyl carnitine) take place outside the mitochondrial barrier and is consistent with some of the evidence obtained on localization of mitochondrial enzymes (Allman et al., 1966, and references cited). The mitochondria from fish skeletal muscle, and particularly those from lateral line muscle, appear to have a greater ability to activate the extramitochondrial fatty acids than do those from the skeletal muscle of rat and other species, which were found to oxidize carnitine ester of fatty acids readily without being able to oxidize free acids to any appreciable extent (Bode and Klingenberg, 1964). The fact that the oxidation of fatty acids by fish mitochondria takes place in the absence of added carnitine (see also Brown and Tappel, 1959; Bilinski and Jonas, 1964) does not necessarily reflect a carnitine independent pathway. Since addition of carnitine stimulates the activity, these data are rather interpreted to signify an endogenous concentration of carnitine below the level required for maximum activity. The carnitine-dependent oxidation of long chain fatty acids is known to occur widely in the animal kingdom, as indicated by the distribution of the enzyme carnitine palmityl transferase, which was also found to be present in cod (Norum and Bremer, 1966).

Though the role of CoA in the sequence of enzymic reactions of fatty acid oxidation is well established (Lynen, 1955), the interpretation of its effect on the oxidative activity of mitochondria is made rather difficult by the evidence suggesting the existence of a mitochondrial barrier not permeable to CoA (Chappell, 1964; Greville, 1966). Under these conditions the CoA supplied to mitochondria in the incubation medium does not appear to be able to participate directly in the oxidative reactions within the mitochondria. In presence of added carnitine, CoA was found to stimulate the oxidation of fatty acids by the various types of fish mitochondria. This might reflect a larger CoA requirement for the initial activation, caused by an increased rate of transfer of fatty acid to the site of oxidation within mitochondria, which takes place in a medium enriched with carnitine. The reason for the difference in the response to CoA of mitochondria from muscle and the other tissues of trout, observed in the absence of added carnitine, is not known. This might be due to various factors, such as differences in the mitochondrial structure or in levels of enzymic activity and endogenous metabolites. It is known that in mammals, mitochondria of various origins may differ significantly in response to cofactors (Bauer and Von Korff, 1967). The addition of CoA (0.1–1.0 mM) was found to have an inhibitory effect on fatty acid oxidation by rat liver homogenates (Ontko and Jackson, 1964; Ontko, 1967) and it was suggested that this could result from reversal of acylcarnitine formation. On the other hand, the addition of CoA (0.5 mM) to rat heart mitochondria was shown to stimulate fatty acid oxidation (Fritz and Yue, 1963). Differences in the structure

of mitochondrial membranes of various organs were suggested by Beenakkers et al. (1967) in relation to the permeability to short chain fatty acids.

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Some Lernaeopodidae (Copepoda) from Fishes of British Columbia

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Nine species of copepods in the family Lernaeopodidae were collected as parasites of teleost or elasmobranch fishes common to coastal waters of British Columbia. One species (*Schistobrachia tertia*) has not been previously described. Two species (*Dendrapta cameroni longiclavata* and *Lernaeopodina pacifica*) are new records for the Canadian fauna. The remaining six include *Pseudocharopinus dentatus*, *Clavella adunca*, and detailed descriptions of *Clavella parva*, *Clavella perfida*, *Nectobranchia indivisa*, and *Brachiella robusta*, the last-named displaying a range of morphological variability hitherto unknown in Lernaeopodidae.

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INTRODUCTION

DURING TRAWLING OPERATIONS of the FRB research vessel *G. B. Reed* in British Columbia waters in May and June 1968, and in May 1969, the majority of fish species caught were examined for the presence of parasitic Copepoda. An interesting collection of these parasites was gathered, the family Lernaeopodidae being represented by nine species. One of these species was still unknown, whereas two others had not been reported from the Canadian Pacific. The existing descriptions of the six species previously known from this region are, in the main, inadequate.

The collection was made off the west coast of Vancouver Island, mainly in the vicinity of La Perouse Bank and at the entrance to Juan de Fuca Strait. Results of examinations of specimens made available to me by Dr J. Holmes, University of Alberta, Edmonton, are also reported. His specimens were collected partly near Friday Harbour (Juan de Fuca Strait) and partly off the west coast of Vancouver Island. All the fishes that harbour the parasites comprising this collection are common throughout the area, so that the distribution of the parasites described can be expected to be equally broad. Consequently, this paper will not include the exact locality of each find and detailed specimen records are not included, except for the newly described species.

The description of each species is followed by comments. There is no separate discussion section. Methods used in the examinations of the specimens were similar to those used in my earlier work (Kabata, 1968).

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DESCRIPTIONS OF SPECIES

Schistobrachia tertia sp.n.

(Fig. 1–16)

Record of specimens — Twenty-five females, mainly ovigerous, one with a male attached, were collected, between May 30 and June 10, from six infected hosts captured off the west coast of Vancouver Island, in the region of La Perouse Bank and at the mouth of Juan de Fuca Strait, at depths ranging from less than 50 to 200 fath. One of these specimens becomes the holotype and three others the paratypes of the species. These specimens are deposited in the National Museum of Natural Sciences, Ottawa, Reg. No. NMC 15068.

Hosts — *Raja rhina* Jordan and Gilbert, 1880; *Raja binoculata* Girard, 1854.

Habitat — Gills and buccal cavity.

Description of the female — The general appearance of *S. tertia* (Fig. 1) gives an impression of slenderness. The cephalothorax is short and has a narrow anterior margin. It widens posteriorly and forms broad "shoulders" with the bases of the second maxillae (Fig. 2). Immediately behind these bases is the narrow beginning of the trunk (Fig. 1, 2). The anterior third of the trunk is narrow and cylindrical, with some transverse wrinkling. From its distal end the trunk widens towards its posterior extremity. The extremity (Fig. 3, 4) is roughly rounded in outline. In dorsal view (Fig. 3) it displays the anal slit (*a*) with the bases of the posterior processes flanking it on either side (*pp*). These processes are about two-thirds as long as the trunk, are cylindrical, and taper to fine points (Fig. 1). The ventral aspect of the posterior extremity (Fig. 4) discloses the absence of a genital process. The attachments of the egg sacs (*es*) can be seen, ventrolateral to the bases of the posterior processes. The dimensions of the female (in mm, based on measurements of six specimens) are as follows: cephalothorax length, 1.70; cephalothorax width, 1.02; trunk length, 6.12–8.50; trunk width, 2.55–3.15; arms length, 9.35; arms width, 0.68; holdfast span, 5.10–5.44; posterior processes length, 4.25–5.10; posterior processes width, 0.68; egg sac length, 6.80–14.45; and egg sac width, 0.85–1.36.

(The length of the second maxillae, the "arms," is very difficult to measure, since these appendages tend to contract or twist, or both, in many preserved specimens.)

The first antenna (Fig. 7, 8) consists of three parts. The proximal part forms about half of the entire appendage and is more robust than the distal half. Near the junction of the two halves, on the dorsomedial side, there is a long and slender seta (Fig. 7). The distal half consists of two parts: a middle and a terminal one. The middle part is short, cylindrical, and provided with a short seta near its junction with the terminal part. The latter is longer, also cylindrical, and provided with the apical armature. The details of the armature (Fig. 8) are similar to those described earlier (Kabata, 1964) for the type species of the genus. The armature comprises three long setae and three short structures, referred to as tubercles. Seta 4 is robust and digitiform; seta 5 is of about the same length but more slender and sharp-pointed; and seta 6 is long and whiplike. Tubercles 1–3 are of about the same shape and size, each of them being spatially associated with the base of one of the setae.

The second antenna (Fig. 9, 10) has a bulbous exopod (*ex*) partly covered with minute denticles. The endopod (Fig. 9) is two-segmented. The distal segment is characterized by a prominent ventral swelling, also bearing many scattered and tiny denticles. Somewhat medial to the apex of the swelling is a short, though apparently strong, seta. Dorsal to the swelling is a large and powerful hook 1 (the curvature of the hook is not obvious in Fig. 9 because of its position). At its base from the medial side is seta 2, slender and shorter than the hook. There is a spiny pad on the basal segment of the endopod and another similar pad on the sympod of the antenna (not seen in Fig. 10).

The mandible (Fig. 11) has a dental formula H1, Z1, H1, Z1, H1, Z1, N5.

The first maxilla (Fig. 12, 13) has three terminal papillae surmounted by sturdy though flexible setae. The exopod is well developed and provided with two setae of unequal length (Fig. 13). The shorter one might be, in fact, simply a process of the exopod, rather than a seta. At

the level of the exopod, the dorsal margin of the appendage is covered with a patch of denticles (Fig. 12). Another similar patch is present near the base of the dorsalmost papilla, on the medial side of the appendage (shown ghosted in Fig. 12).

The second maxilla (Fig. 1, 5) is long and slender. In some specimens it is clearly longer than the trunk. The two second maxillae are separate throughout, being only lightly linked at their tips by the vestigial bulla. The attachment of the parasite has been taken over by a unique holdfast (Fig. 5), formed by modification of the tips of both second maxillae. The holdfast has three pairs of tines. The dorsal pair (*d*) is short, blunt, and conical, projecting laterally from the tips of the appendages. Immediately distal to this pair both tips constrict abruptly, each one then expanding into two processes (*v*, Fig. 1, 5). The latter vary somewhat in length and sometimes in shape, depending on the conditions at the site of attachment. They are, however, invariably present.

The maxilliped (Fig. 14–16) is distinguished by a small swelling on the medial margin. Near the base of the swelling is a slender seta arising from a papilla (Fig. 15). There is also a small patch of tiny denticles on the medial margin, in the axilla formed by the appendage with the subchela (Fig. 14). The claw of the subchela is provided with a secondary tooth (*st*, Fig. 16). There is the usual auxiliary spine (*ar*) at the base of the claw. Near the base of the spine there is also a short papilla which appears to carry a number of minute denticles. The inner margin of the subchela is also lightly armed with several denticles (Fig. 16).

The male (Fig. 6) has not been examined in detail. Its general structure resembles that of the males in all genera belonging to the so-called *Charopinus* group (Kabata, 1964).

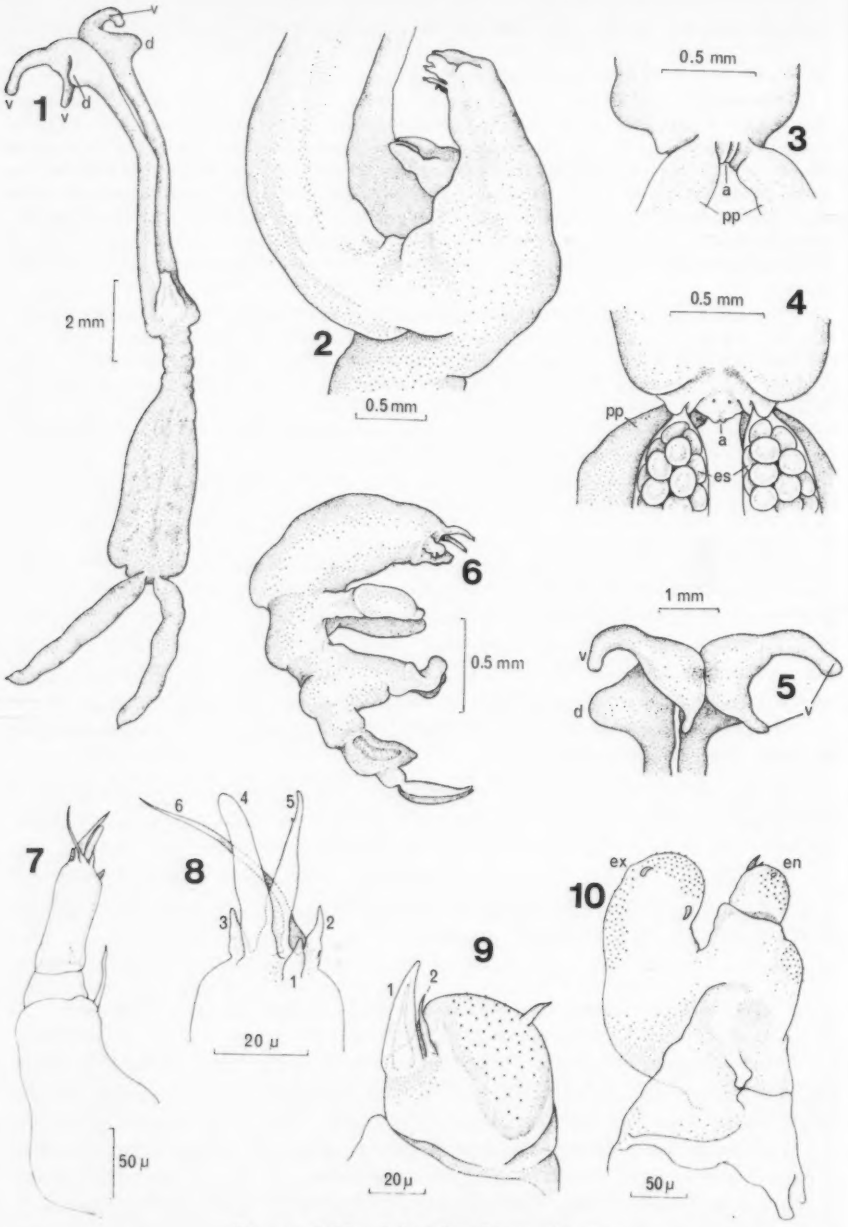
Comments — The genus *Schistobrachia* Kabata, 1964, has two other known species: *S. ramosa* (Kroyer, 1863) and *S. chimaerae* (Yamaguti, 1939). Both species have holdfasts with only two pairs of prongs and of different general shape. In particular, they are devoid of the constriction present in the holdfast of *S. tertia*, between the conical dorsal tines and the two ventral pairs. The method of attachment is also different. The holdfast of *S. tertia* is a simple anchor, not capable of encircling the gill arch of the host in the manner typical of *S. ramosa*.

In the two other species of *Schistobrachia* the perianal area is raised above the surface of the posterior extremity in a definite swelling (cf. Kabata, 1964, fig. 68). No such swelling is present in *S. tertia* (see Fig. 3).

Judging by the abundance of specimens, *S. tertia* is a species common in the waters of British Columbia. It is possible that it occurs on most species of *Raja* in this and adjacent areas.

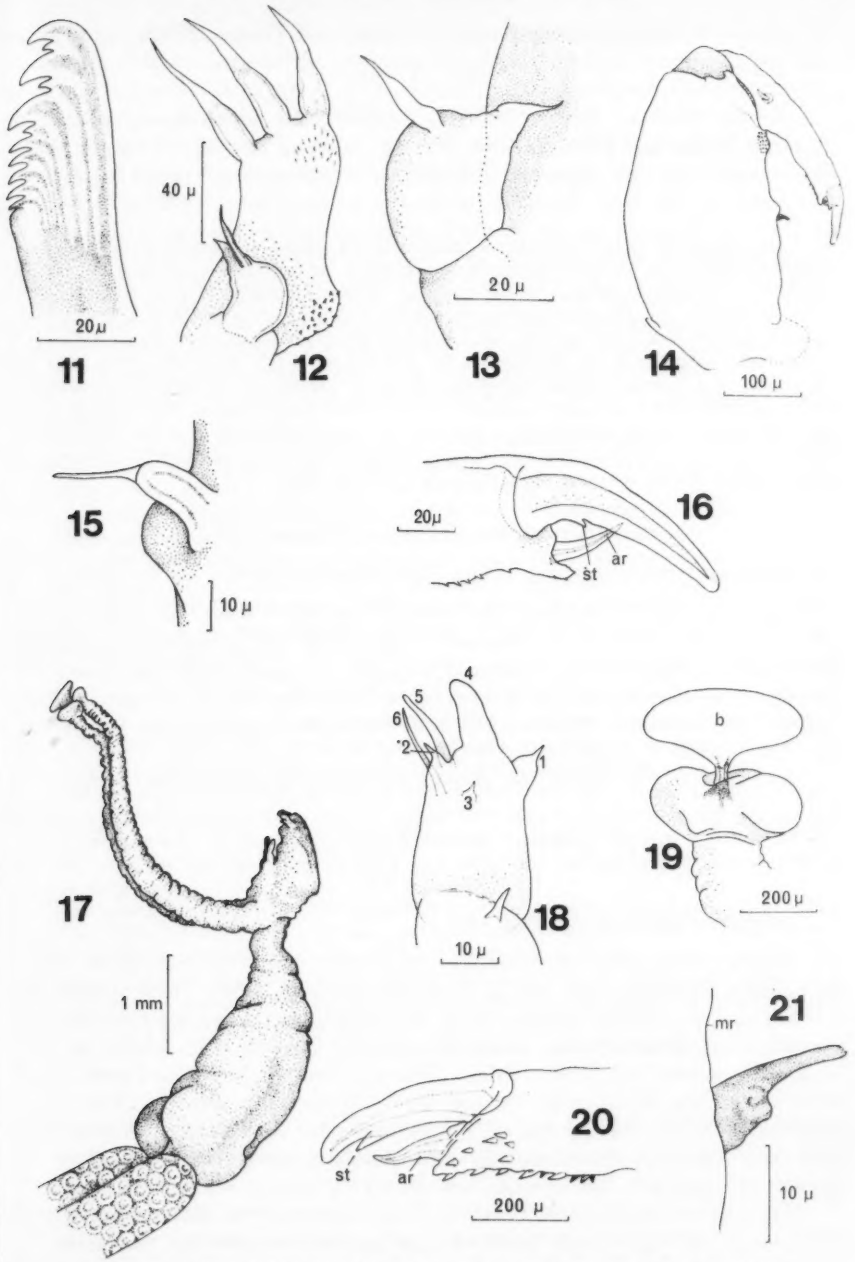
Dendrapta cameroni longiclavata Kabata and Gusev, 1966

One ovigerous female of this parasite was found on the skin of *Raja inornata* Jordan and Gilbert, 1880. The find constitutes a new record for the Canadian fauna. This subspecies was previously described from the region of the Kamchatkan Peninsula and Sakhalin, where it parasitizes the skin of *Raja kenojei*, *Raja smirnovi*, and an unspecified species of the same genus (Kabata and Gusev, 1966). The original *D. cameroni* (Heller, 1949) was found in the Canadian Atlantic. The genus is absent from the European side of the Atlantic.



FIGURES 1–10. ***Schistobrachia tertia,*** sp.n.

Fig. 1. Female, entire, dorsal; Fig. 2. Cephalothorax, lateral; Fig. 3. Posterior extremity, dorsal; Fig. 4. Same, ventral; Fig. 5. Attachment organ, ventrolateral; Fig. 6. Male, entire, lateral; Fig. 7. Female, first antenna, entire, dorsal; Fig. 8. Same, tip, medial; Fig. 9. Second antenna, endopod, lateral; Fig. 10. Same, entire, medial.



FIGURES 11–21. ***Schistobrachia tertia,*** sp.n., and ***Lernaeopodina pacifica.***
Fig. 11. *Schistobrachia tertia,* mandible, distal part, lateral; Fig. 12. First maxilla, distal part, lateral; Fig. 13. Same, exopod, ventrolateral; Fig. 14. Maxilliped, entire, dorsal; Fig. 15. Same, armature of medial margin, ventral; Fig. 16. Same, claw of subchela, ventral; Fig. 17. *Lernaeopodina pacifica,* female, entire, lateral; Fig. 18. First antenna, tip, medial; Fig. 19. Bulla, lateral; Fig. 20. Maxilliped, claw of subchela, dorsal; Fig. 21. Same, armature of medial margin, lateral.

Lernaeopodina pacifica Kabata and Gusev, 1966

(Fig. 17–21)

Seven ovigerous specimens of *L. pacifica* were taken from the gills of *R. rhina* Jordan and Gilbert, 1880. This find is also a new record for the Canadian fauna. The only previous find of this parasite was recorded by Kabata and Gusev (1966) from Sakhalin, where it was found on the gills of *R. kenojei*.

The original description included a drawing of the general appearance of a young, non-ovigerous female. The specimen illustrated was probably not fully grown and its trunk was disproportionately short in relation to the length of its second maxillae. A fully adult female is shown here in Fig. 17. The specimen was relaxed before fixing and the length of its arms is probably not unlike that of living parasites. Some details of the original description require corrections. A short seta is present at the distal end of the central part of the first antenna (Fig. 18). The bulla possesses a short but distinct manubrium (Fig. 19). The claw of the subchela of the maxilliped is provided with a secondary tooth (*st*, Fig. 20). (Kabata and Gusev (1966) show this claw with the secondary tooth broken off, as indicated by a small projection from the margin of the claw.) The spine of the medial margin of the maxilliped (Fig. 21) is characteristic, showing an abrupt change in the diameter some distance from the base.

Pseudocharopinus dentatus (Wilson, 1912)

Synonyms — Brachiella dentata Wilson, 1912; *Charopinus dentatus* (Wilson, 1912).

Numerous adult females of this species were taken from the buccal cavity, gills, skin, and claspers of *R. rhina* and *R. binoculata*. The parasite was originally discovered in this area by Wilson (1912) and was later recorded from it by Fraser (1920). Further south, Wilson found it off the coast of Oregon (Wilson, 1935) and California (Wilson, 1922). A detailed description of the female has been given by Kabata (1964).

The find of this species on *R. rhina* constitutes a new host record.

Clavella parva Wilson, 1912

(Fig. 22–33)

Synonym — Clavella recta Wilson, 1915.

Several adult and juvenile females were taken from the fin rays of *Sebastodes alutus* (Gilbert, 1890) and *S. diploproa* (Gilbert, 1890). Both species are new hosts. The original type host is *S. auriculatus*. Later records mention this parasite on *S. melanops* and *Sebastodes* sp. (cf. Wilson, 1915, 1922), as well as *S. taczanowskii* (cf. Gusev, 1951), from the Asian side of the Pacific. The latter record was made under the synonym *Clavella recta*. Fraser (1920) identified fish of other families also as alleged hosts of this parasite. He mentions *Embiotoca lateralis* (Embiotocidae) and *Artedius harringtoni* (Cottidae) as carrying the parasite, but he does not describe it nor give any drawings.

The original description (Wilson, 1912) included the illustration of the first maxilla in which both terminal papillae were topped by two setae, a condition unique in Lernaeopodidae. Reexamination of the type material,

kindly made available by Dr Cressey from the Smithsonian Institution, failed to confirm this finding. Since the presence of this double-seta condition was the main difference between *C. parva* and *C. recta*, I propose to relegate the latter to synonymy with the former.

In view of many doubts that have been created by the brevity of the original description of *C. parva*, it is advisable to redescribe it in some detail.

The general appearance is illustrated in Fig. 22 and 23. A juvenile female is shown in Fig. 24. In the adult, the cephalothorax is as long as, or longer than, the trunk. The trunk is roughly quadrangular, with a slightly projecting perianal area in the centre of the posterior margin (Fig. 22, 28, 29). The ventral aspect shows a short genital process (*gp*, Fig. 29), as well as oblique slits accommodating the openings of the oviducts. The young females have disproportionately small trunks and long cephalothoraces. The dimensions of six specimens from the type material (in mm) are as follows: cephalothorax length, 1.40–1.50; cephalothorax width, 0.30–0.32; trunk length, 0.96–1.28; trunk width, 0.80–1.04; egg sac length, 1.00–1.20; and egg sac width, 0.36–0.50.

The first antenna (Fig. 25) is distinguished by the reduction in the number of structures comprising its apical armature. Only the digitiform seta 4 and the typical bifid seta 5 are present in addition to the tubercle 1. The tubercle can be here compared rather to a short and robust spine. In all other respects, this appendage resembles that of all other species of *Clavella*.

The second antenna (Fig. 26, 27) is subdivided apically into two lobes, one of which appears to be unarmed, whereas the other carries two setae, one much smaller than the other. A large and thick seta is present at the base of these two lobes. At about the same level, the tip carries a small patch of about six setules. Some minuscule denticles might be present at the tips of both lobes. At the base of the appendage the anterolateral corner of the cephalothorax is provided with two characteristic papillae (*p*, Fig. 26).

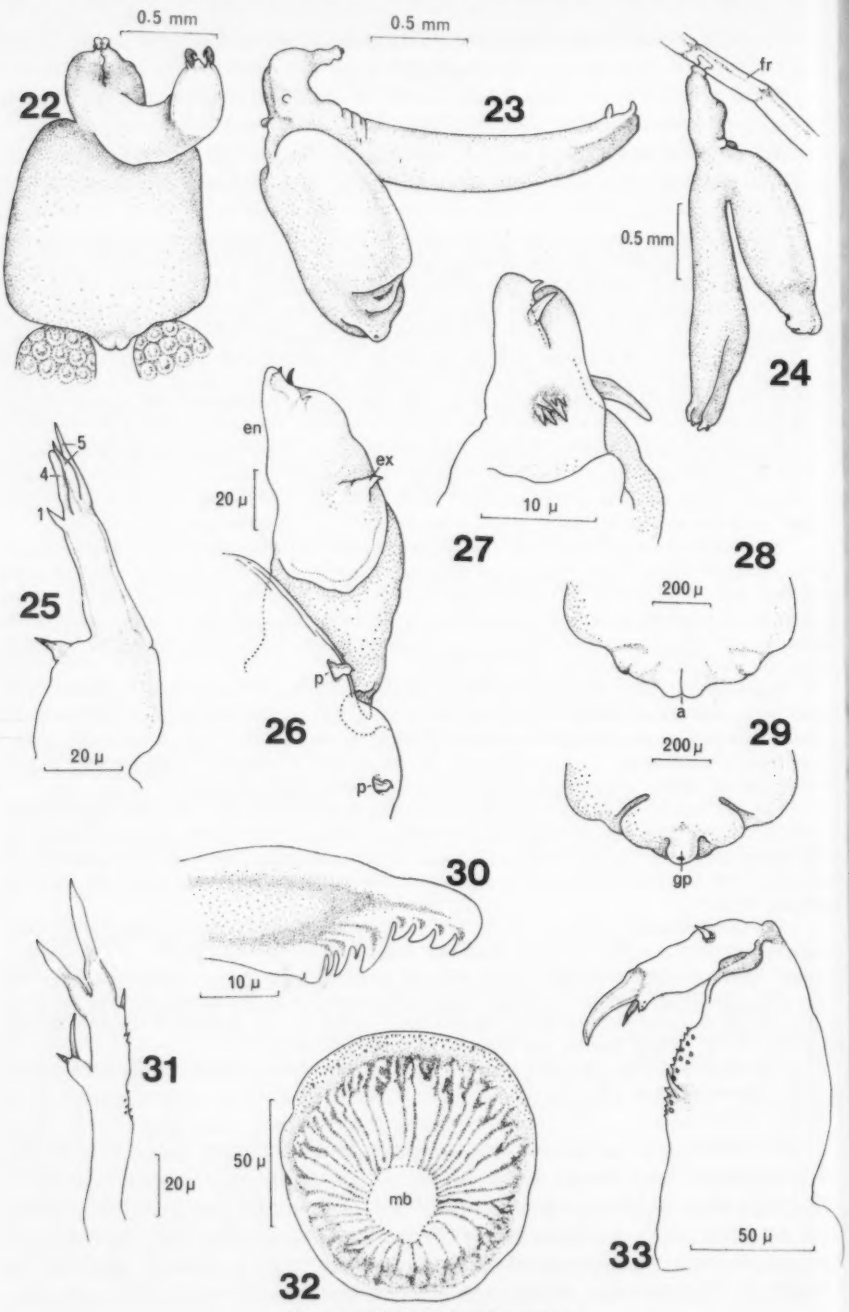
The mandible (Fig. 30) has a dentiferous margin with teeth divided into two series. The distal series consists of four primary teeth of about equal size and similar shape, whereas the proximal one consists of a large primary tooth, followed by one secondary and two long and narrow basal teeth (Nebenzähne). The two series are set off from each other by an abrupt widening of the mandibular blade at the level of the first tooth of the proximal series.

The first maxilla (Fig. 31), contrary to the original description, has terminal papillae each carrying a single, thick seta. A small seta is present at the base of the dorsal papilla. Two groups of denticles are present on the dorsal margin of the appendage, one at the level of the exopod and the other near the tip. The exopod is prominent, cylindrical, and equipped with two setae of unequal length.

The second maxilla (Fig. 22, 23) in most adult females shows a characteristic flexion, particularly well shown in Fig. 23. It is short and fused with its opposite number. Small nipples, present on the ventrolateral sides near the bases, carry the openings of excretory ducts. The bulla (Fig. 32) is small. Invariably injected into the fin rays (*fr*, Fig. 24), it has a short manubrium and a flat circular disc with radially disposed ducts within it. The position of the manubrium (*mb*, Fig. 32) is usually just to one side of the centre of the disc.

The maxilliped (Fig. 33) has a large group of denticles on the medial margin. A short seta is also present in that area.

Comments — Clearly, the original description (Wilson, 1912) was based on an abnormal specimen, perhaps one in a state of partial moult. In spite of careful examination, no specimen of the type material was found to possess first maxillae with double setae on their terminal papillae. The presence of the double seta would necessitate serious taxonomic reappraisal of *Clavella*, so that its absence was ascertained with some relief. *Clavella parva* can now



FIGURES 22–33. ***Clavella parva.***

Fig. 22. Female, entire, dorsal; Fig. 23. Same, lateral; Fig. 24. Juvenile female, lateral; Fig. 25. First antenna, entire, ventral; Fig. 26. Second antenna, entire, dorsal; Fig. 27. Same, tip of endopod, ventral; Fig. 28. Posterior extremity of trunk, dorsal; Fig. 29. Same, ventral; Fig. 30. Mandible, distal part, lateral; Fig. 31. First maxilla, lateral; Fig. 32. Disc of bulla, proximal surface; Fig. 33. Maxilliped, entire, lateral.

be considered a normal member of its genus, related within it to the group typified by *C. gracilis* Hansen, 1923 (cf. Kabata, 1963a).

Clavella adunca (Strøm, 1762)

(Fig. 41)

Synonym — Clavella uncinata (Müller, 1776).

Numerous specimens were taken from the gills and branchial cavity of *Gadus macrocephalus* Tilesius, 1810, and *Microgadus proximus* (Girard, 1854). This species is nearly ubiquitous, having been recorded from both the Atlantic and the Pacific oceans, where it parasitizes many host species. Several authors found it in the eastern North Pacific, parasitic on *G. macrocephalus*, *Hexagrammos octogrammus*, and *Pleurogrammus* sp. A detailed description of the species has been given by Kabata (1963b).

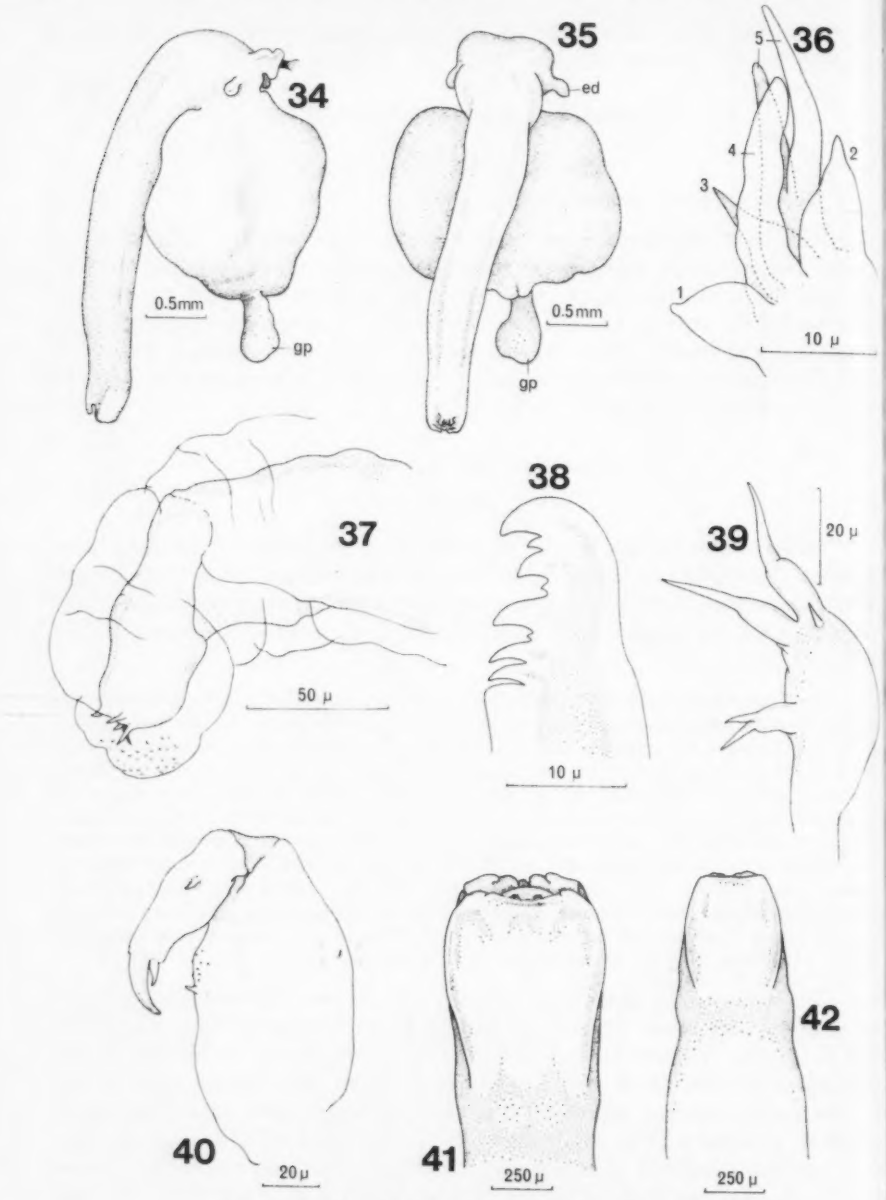
Clavella perfida Wilson, 1915

(Fig. 34–40, 42)

Four females of this species were found on the gills of *Theragra chalcogramma* (Pallas, 1811). Since the previous records (Wilson, 1915, 1920; Gusev, 1951; Markevich, 1956) were not accompanied by accurate descriptions and since this species bears a close resemblance to *C. adunca*, its reexamination is worthwhile.

The general appearance of *C. perfida* is shown in Fig. 34 and 35. The main features that distinguish it from *C. adunca* are its large genital process (*gp*, Fig. 34, 35) and the tapering apex of its cephalothorax. Its appendages are virtually identical with those of *C. adunca*. The tip of the first antenna (Fig. 36) has the full complement of structures also occurring in *C. adunca*; the second antennae (Fig. 37) of the two species are indistinguishable. The same is true of the mandibles (Fig. 38). The first maxilla of *C. perfida* (Fig. 39) is devoid of denticles on its dorsal margin, in contrast to the corresponding appendage of *C. adunca*, invariably provided with them. The nipples on the second maxilla of *C. perfida* (Fig. 34, 35) are much more prominent than the corresponding structures in *C. adunca*. No distinguishing features can be found in the maxilliped (Fig. 40), unless one takes into account the fact that the spinulation of the medial margin is rather weaker than in *C. adunca* and that the inner margin of the subchela appears to be completely lacking in spinules that are always present in *C. adunca*.

Comments — The shape and relative size of the cephalothorax provide the quickest and most dependable way of distinguishing between *C. adunca* and *C. perfida*. Figures 41 and 42 show the dorsal views of the ends of the cephalothoraces of these two species respectively. The figures were drawn to the same scale and represent specimens of comparable sizes. The dorsal shield of *C. adunca* (Fig. 41) is about twice the length of that of *C. perfida*. In *C. adunca* the shield is broadest in its anterior half and becomes narrower posteriorly, whereas in *C. perfida* its greatest width is at about midlength and the shield tapers in both directions. The differences in the sizes of the dorsal shields are reflected in similar differences in all the appendages. *Clavella perfida* has them only half as large as those of a specimen of *C. adunca* of similar size.



FIGURES 34–42. ***Clavella perfida*** and ***Clavella adunca.***

Fig. 34. *Clavella perfida*, female, entire, lateral; Fig. 35. Same, dorsal; Fig. 36. First antenna, tip, ventromedial; Fig. 37. Second antenna, ventral; Fig. 38. Mandible, tip, lateral; Fig. 39. First maxilla, lateral; Fig. 40. Maxilliped, ventral; Fig. 41. *Clavella adunca*, dorsal shield; Fig. 42. *Clavella perfida*, dorsal shield.

In an earlier publication (Kabata and Gusev, 1966), I expressed the view that a careful examination would probably result in the transfer of *C. perfida* to synonymy with *C. adunca*. This opinion must now be reversed. It seems certain that *C. perfida* is a valid species. It is worth adding here also, that this species is restricted to *T. chalcogramma*, a host on which no authenticated occurrence of *C. adunca* has ever been reported.

Brachiella robusta (Wilson, 1912) comb.n.

(Fig. 43–73)

Synonyms — *Clavella robusta* Wilson, 1912; *Clavellopsis robusta* (Wilson, 1912).

Described originally as *Clavella* (cf. Wilson, 1912), this parasite, because of the structure of its second antenna, was subsequently transferred by the discoverer to a newly established genus, *Clavellopsis* (cf. Wilson, 1915). The transfer is tentative, the final decision on the generic identity being reserved until the male has been found.

The type host is *Sebastodes auriculatus*. A later record (Fraser, 1920) extended the host range by including in it *S. pinniger* and *S. melanops*. Fraser observed also that the specimens from the latter host were distinguished by longer posterior processes. Bere (1936) and Pearse (1952) recorded this species from the Atlantic coast of the USA, where they parasitized "mullets." Examination of specimens deposited by these two authors in the Smithsonian Institution, Washington, D.C., shows that they are not conspecific with *B. robusta*. Although they bear general resemblance to this species, they are readily distinguished from it by the absence of a transverse welt on the cephalothorax and by the structure of the bulla. Their specific identity is now being investigated.

In the course of this study numerous specimens of this parasite have been taken from 13 species of *Sebastodes*. Many females were found with males attached. The morphology of the male was not studied in detail, but its general structure (Fig. 65) places this species beyond doubt in the genus *Brachiella*.

The examination of a rich collection of *B. robusta* has revealed some facts that are unique among Lernaeopodidae. They also contribute to the general knowledge of parasitic Copepoda. It was, therefore, considered desirable to describe the parasite in detail.

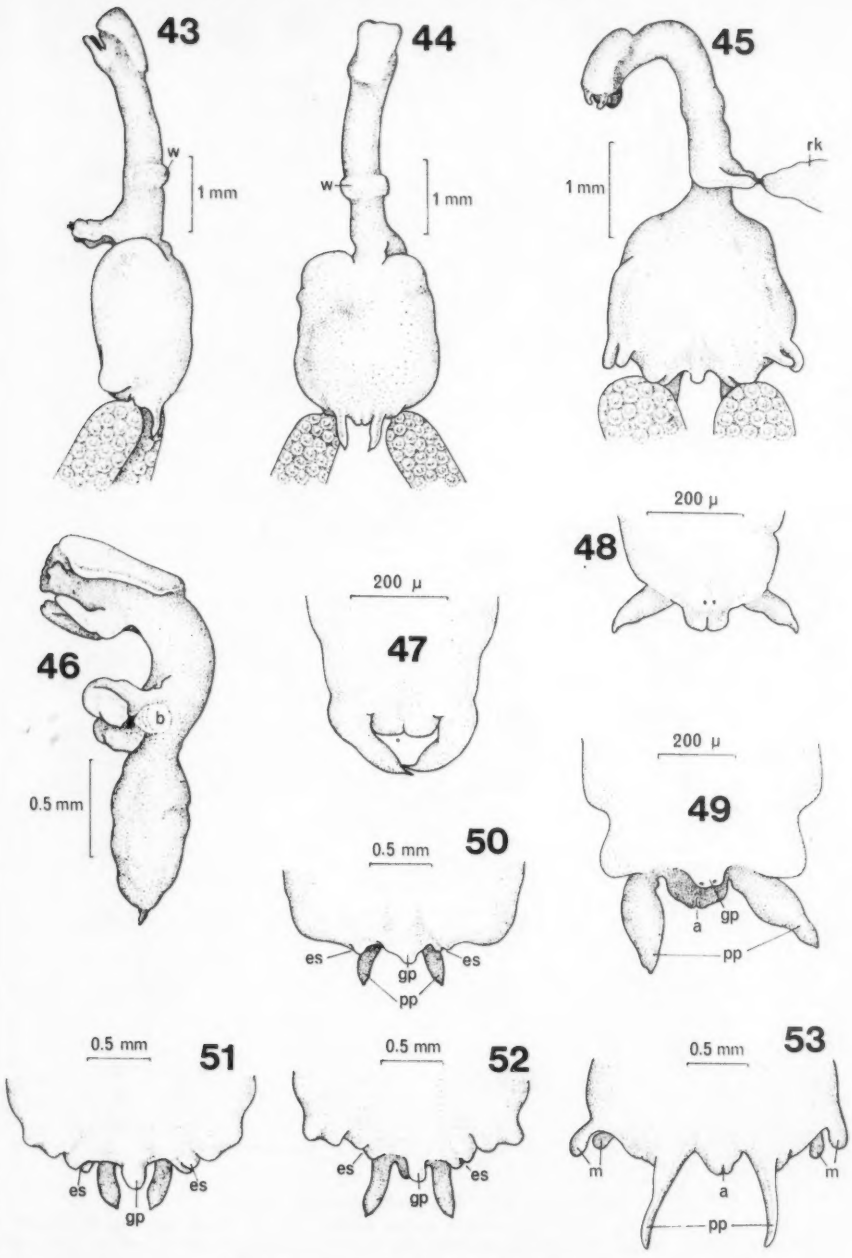
Description of the female — The general appearance of *B. robusta* is characterized by a range of morphological variability unparalleled among Lernaeopodidae. The range is so extensive that the examination of a less abundant material would have probably resulted in description of at least three species. There are three distinct morphological forms, linked together by many intermediate characteristics. The first of them corresponds with the original description and is referred to here as *B. robusta f. robusta* (Fig. 43–45). Its cephalothorax is elongated and cylindrical. The dorsal shield (Fig. 54) has a truncated anterior margin and narrows posteriorly. A transverse welt occurs on the dorsal wall of this part of the body, about one-third of its length from the base (*w*, Fig. 43, 44). This feature is rare among *Brachiella* (it occurs in *B. tetrici* Kabata, 1968), but

is found in *Epibrachiella*. Directly behind the bases of the second maxillae, the trunk has a short, cylindrical neck (Fig. 44), which abruptly flares out into the main part of the trunk. The trunk is roughly rectangular, with rounded corners. In some specimens the posterior margin is wider than the anterior one (Fig. 45). It is the posterior extremity, however, that shows the greatest range of variations. The main feature of this extremity is a pair of posterior processes, situated one each next to the perianal swelling and dorsal to the bases of the egg sacs (Fig. 43). The processes are formed by modification of the caudal rami, as can be seen by examining a series of young females of increasing trunk lengths. Figure 46 shows such a female with trunk length 0.9 mm. Figure 47 shows its posterior extremity, clearly demonstrating the nature of the posterior processes. A ventral view of a female with trunk length 1.0 mm is shown in Fig. 48. Two black dots mark the positions of the genital orifices. With subsequent development the area around them forms a digitiform genital process that becomes quite long in some specimens. The posterior extremity of a young female with trunk length 1.4 mm is shown in Fig. 49. The genital process is now quite definite, the perianal area (*a*) forms a definite swelling and the posterolateral corners of the trunk begin their lateral expansion. By the time the female has become fully mature (Fig. 50), the broadness of the trunk overshadows the posterior processes. In Fig. 50, the posterolateral corners of the trunk are smoothly rounded. In another specimen (Fig. 51) they show slight indentations; in yet another (Fig. 52) the indentations become more marked, suggesting an incipient division of the corners into two lobes. The genital process (*gp*) has now become prominent, as has also the perianal area. Figure 53 shows another stage in this range. The process of lobation of the posterolateral corners has resulted in the formation of a pair of processes (*m*) on each corner. The processes are short, stubby, often pedunculate. In this form the posterior processes (*pp*, Fig. 53) tend to be longer and more slender than in the forms with less deeply cleft posterolateral corners.

The second morphological form has been named *B. robusta f. longidigita*. Its general appearance is illustrated in Fig. 66–69. It differs from the preceding form essentially in the structure of the trunk. The anterolateral corners form rounded outgrowths (*o*, Fig. 66, 69), demarcated from the rest by a slight constriction. The posterolateral corners have each a pair of processes (*m*, Fig. 66, 69), far longer than any structure encountered in the preceding form. That the length of these processes cannot be considered simply as an age effect can be seen in Fig. 69, showing the trunk of a young female (length 0.85 mm). At this stage the posterior extremity of *B. robusta f. robusta* (Fig. 49) has no traces of any posterolateral outgrowths. A truly remarkable feature, however, is the presence between the posterior processes of a long, cylindrical process that must be regarded as the abdomen, since it carries the anal orifice at its tip (*a*, Fig. 66). No abdomen has hitherto been found in Lernaeopodidae. The length of the genital process is also quite considerable, though in those specimens that are attached to the tips of gill rakers the genital processes may be quite small. (The region of the process pushes hard against the surface of the raker.) Figures 67 and 68 show specimens attached in this manner. Their posterolateral processes are about as long as the trunk itself.

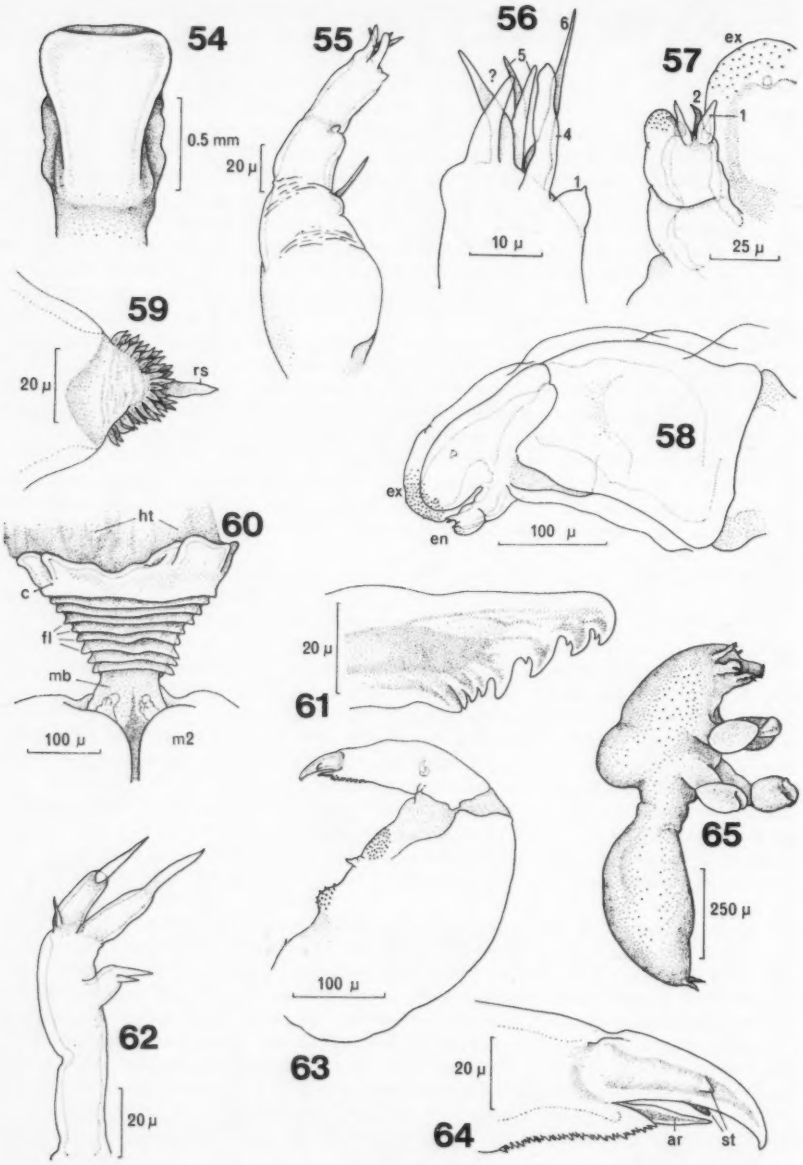
The third form has been named *B. robusta f. tuberculata*. Like the preceding form, it has well-developed posterolateral processes (*m*, Fig. 70–73), though they tend to be shorter and more stubby than those of *B. robusta f. longidigita*. Sometimes they might be pedunculate or show slightly lobate tips. The abdomen and the genital process are also shorter than in that form. The main distinguishing feature of *B. robusta f. tuberculata* is the lateral margins of the trunk, provided with another pair of outgrowths, situated between the anterior process (*o*) and the posterolateral pair (*m*). These processes are labelled (*r*) and (*s*) in Fig. 70–73. Together with (*o*) and (*m*), they impart to the trunk margins a tuberculate appearance that suggested the name for this form. The anterior process (*o*) is subdivided, consisting of a ventral and a dorsal component (Fig. 71). All processses have been found in the smallest specimen available for examination (trunk length 1 mm).

The size of the parasite appears to be host dependent. Taking all specimens together (about 100 measured), the ovigerous females have the following dimensions (in mm): cephalothorax length, 2.20–3.20; cephalothorax width, 0.40–0.68; trunk length, 1.12–2.60; and trunk width, 1.00–2.40.



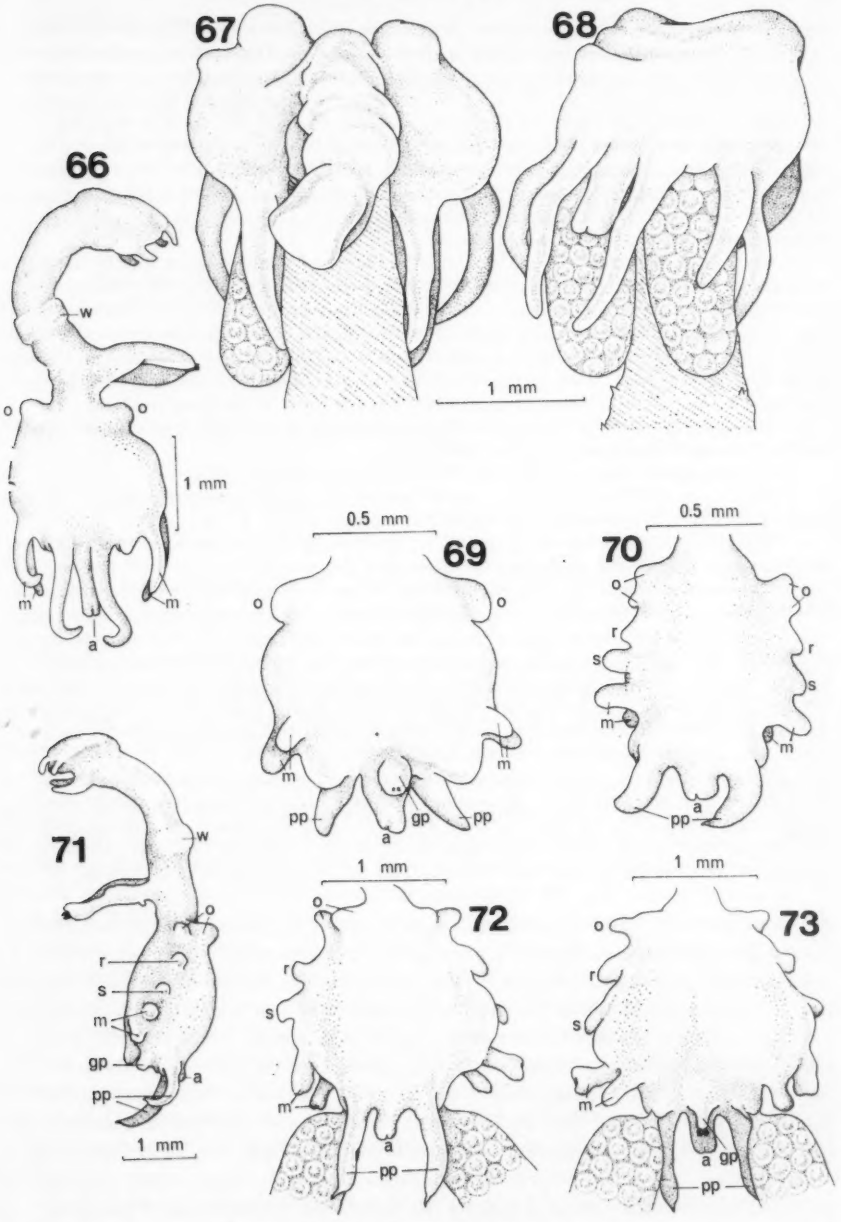
FIGURES 43–53. ***Brachiella robusta*** comb.n.

Fig. 43. *B. robusta f. robusta*, female, entire, lateral; Fig. 44. Same, dorsal; Fig. 45. Same, ventral; Fig. 46. Juvenile female (trunk length 0.9 mm), lateral; Fig. 47. Same, posterior extremity, dorsal; Fig. 48. Juvenile female (trunk length 1.0 mm), posterior extremity, ventral; Fig. 49. Juvenile female (trunk length 1.4 mm), posterior extremity, ventral; Fig. 50. Adult female, posterior extremity, ventral; Fig. 51 and 52. Same; Fig. 53. Same, dorsal.



FIGURES 54–65. ***Brachiella robusta*** comb.n.

Fig. 54. Dorsal shield; Fig. 55. First antenna, entire, medial; Fig. 56. Same, tip; Fig. 57. Second antenna, endopod, lateral; Fig. 58. Same, entire; Fig. 59. Upper lip, external surface; Fig. 60. Bulla, anterior; Fig. 61. Mandible, distal part, lateral; Fig. 62. First maxilla, lateral; Fig. 63. Maxilliped, dorsal; Fig. 64. Same, claw of subchela, dorsal; Fig. 65. Male, entire, lateral.



FIGURES 66–73. ***Brachiella robusta*** comb.n.

Fig. 66. *B. robusta f. longidigita*, female, entire, dorsal; Fig. 67. Same, ventral; Fig. 68. Same, dorsal; Fig. 69. Trunk of juvenile female (trunk length 0.85 mm), ventral; Fig. 70. *B. robusta f. tuberculata*, trunk of juvenile female (trunk length 1.0 mm), ventral; Fig. 71. Adult female, entire, lateral; Fig. 72. Trunk of adult female, dorsal; Fig. 73. Same, ventral.

The structure of the appendages is uniform throughout the species. I found no differences in any characters among the three morphological trunk forms. This uniformity extended to the structure of the bulla, a characteristic organ, unlike any previously described in Lernaeopodidae.

The first antenna (Fig. 55, 56) is indistinctly segmented, its proximal half slightly inflated. At the tip of this half, the dorsomedial side carries a long seta (Fig. 55). Signs of subdivision into two are present in the distal half. Another, smaller seta is present at the level of possible subdivision. The apical armature (Fig. 56) includes the usual three long setae: the digitiform seta 4, the bifid seta 5, and the long seta 6. The conical tubercle 1 is also present at the base of seta 4. In addition, two structures of uncertain homology are present, (*?*, Fig. 56). It is possible that they represent modifications of the usual tubercles 2 and 3.

The second antenna (Fig. 57, 58) is typical for the genus. The exopod (*ex*, Fig. 58) is covered with minute denticles. The distal segment of a two-segmented endopod (Fig. 57) is equipped with a typical armature. A conical swelling, carrying many tiny denticles, can be found on the ventral side. A sturdy spine 1 corresponds to the hook, normally present in more primitive genera in this position. At the base of the spine is the auxiliary seta 2. There are two more setae at the base of the ventral outgrowth. These might be homologous with processes 3 and 4 of other Lernaeopodidae (in Fig. 57 one of them is obscured by the other and by the outgrowth).

The upper lip (Fig. 59) has foliate processes arranged in marginal series on both sides of its tip. The tip is prolonged in a flat rostrum (*rs*).

The mandibular formula is H1, Z1, H1, Z1, H1, Z1, N4 (Fig. 61).

The first maxilla (Fig. 62) has an exopod carrying two setae of unequal length. It appears to be devoid of any denticulation of the dorsal margin.

The second maxilla (Fig. 43, 45, 46, 66, 71) is short and separate from its opposite number throughout its length. The nipple with the opening of the excretory duct is small and might be absent in some specimens. The most characteristic feature is the structure of the bulla (Fig. 60). The manubrium (*mb*) is very short and expands gradually into a cup-shaped structure (*c*), thin-walled and fused intimately with the adjacent host tissue (*ht*). Figure 60 shows signs of damage inflicted on the rim of the cup in the course of dissection. The external wall of the cup, just below the rim, carries a series of flanges (*fl*) running horizontally right around it. The series ends near the base of the short manubrium and consists of about eight flanges, partly overlapping one another. Their free edges are directed towards the base of the bulla, probably increasing the security of the parasite's attachment to the host.

The maxilliped (Fig. 63, 64) is short and robust. Its medial margin bears a small seta, situated between two patches of denticles. The proximal patch is on a small swelling and its denticles appear slightly larger than those of the distal patch (Fig. 63). The claw of the subchela (Fig. 64) has two secondary teeth (*st*). The usual auxiliary spine (*ar*) is present. The inner margin of the subchela is equipped with many small denticles that run along one-third of its length.

Comments — Investigation of the structure of *B. robusta* with the abundant material available has disclosed a range of morphological variability hitherto unsuspected in Lernaeopodidae. This raises the question of the validity of many species, established on few specimens and bearing some resemblance to other species, known from equally scanty material. For example, Kabata and Gusev (1966) described some morphological variations in *Eubrachiella gaini* (Quidor, 1913), designated it as *E. gaini dorsituberculata*, and accorded it a subspecific status. The relationship of this form to *Eubrachiella sublobulata* Barnard, 1955, was suggested. An extensive collection might bring to light a continuous range of intermediate stages between these forms and result in a large-scale taxonomic regrouping. In the absence of such material, however, there is no alternative to careful descriptions of available specimens with taxonomic decisions based on these descriptions.

Brachiella robusta is the only species of its genus with posterior processes dorsal to the bases of the egg sacs. It had been hitherto supposed (Kabata, 1966) that the only Lernaeopodidae with posterior processes in this position belonged to the *Charopinus* group.

It is interesting to look at the possibility of links between the morphological type and the host species of *B. robusta*. The table below lists the hosts (genus *Sebastodes*) of the three forms (as represented in the material studied) and the depth range of these hosts.

Morphological type	Host species	Depth range (*fath*)
B. robusta f. robusta	*S. maliger*	<50
	S. caurinus	<50
	S. pinniger	50–150
B. robusta f. longidigita	*S. pinniger*	50–150
	S. crameri	100–300
	S. proriger	50–150
	S. melanops	50–250
	S. alutus	50–300
	S. flavidus	50–150
	S. ruberrimus	50–200
	S. rubrivinctus	50–300
	S. zacentrus	100–200
B. robusta f. tuberculata	*S. aurora*	220–300
	S. diploproa	100–300

The depth distribution range for various species of *Sebastodes* has been compiled from the information obtained by catches of these species during the cruises of *G. B. Reed* when the parasites were collected. Additional information was taken from the work of Alverson et al. (1964).

Brachiella robusta f. robusta appears to occur predominantly on species living in shallow, inshore waters. *Sebastodes pinniger*, which is often encountered in these waters, also harbours the same form, in addition to *B. robusta f. longidigita*. In deeper water the latter form is found on *S. pinniger*. Most other species occurring in bulk in waters deeper than 50 fath are infected by *S. robusta f. longidigita*. At the other end of the distribution range seems to be *B. robusta f. tuberculata*, parasitic mainly on *S. aurora*. The only specimen of *S. diploproa* examined also carried this form. Although at this stage no definite conclusion can be formulated, the shallow-water species, intermediate-depth species, and deepwater species all seem to be associated with a distinct type of *B. robusta*. *Brachiella robusta f. tuberculata*, a form that is more distinct from the other forms than these forms from each other, occurs on *S. aurora*. Study of the taxonomy of *Sebastodes* with the aid of protein electrophoresis (Tsuyuki et al., 1968) suggests that this species is also an odd one within its genus.

Judging from the multiplicity of its closely related and morphologically similar species, *Sebastodes* is in the process of active speciation. One of the mechanisms of this process might be the preferred depth of some *Sebastodes*.

The pressures of speciation of the host fish might also be reflected in the morphology of the parasite, gradual differentiation leading to the process of splitting into several species. If this is so, then the form *B. robusta f. longidigita* could be regarded as being the most primitive of the three. It is equipped with the best developed posterolateral processes, a feature belonging to the most common characteristics of the genus *Brachiella*. It is found on the deeper dwelling hosts; it is probable that the intrusion into the inshore areas is a secondary feature for *Sebastodes*, essentially an open-sea fish. The evolution of the shallow-water habits of the host and the reduction of the posterolateral processes of the parasite could be seen as parallel to each other.

Another unusual feature of *B. robusta* is the number of males; found attached to the females. Most lernaeopodid males are almost ephemeral in the brevity of their lives. Many species have no known males; even more are characterized by scarcity of males. A check on 279 females of *B. robusta* showed that 113 of them had males attached. One had two males. The attachment positions of 107 males were noted. As many as 73 of them were attached to the dorsal shield (usually its lateral margins). Of the remainder, 12 were found on the cephalothorax posterior to the dorsal shield, 3 on the second maxillae, 2 on the margin of the trunk, and 1 on its ventral surface. Only 16 were attached to the genital process. This is a complete reversal of the usual pattern of attachment of the lernaeopodid males. In most species of this family, they attach themselves predominantly to the genital process or near it. It is impossible to say at the moment what biological significance can be attached to this unusual type of male attachment.

Nectobranchia indivisa Fraser, 1920

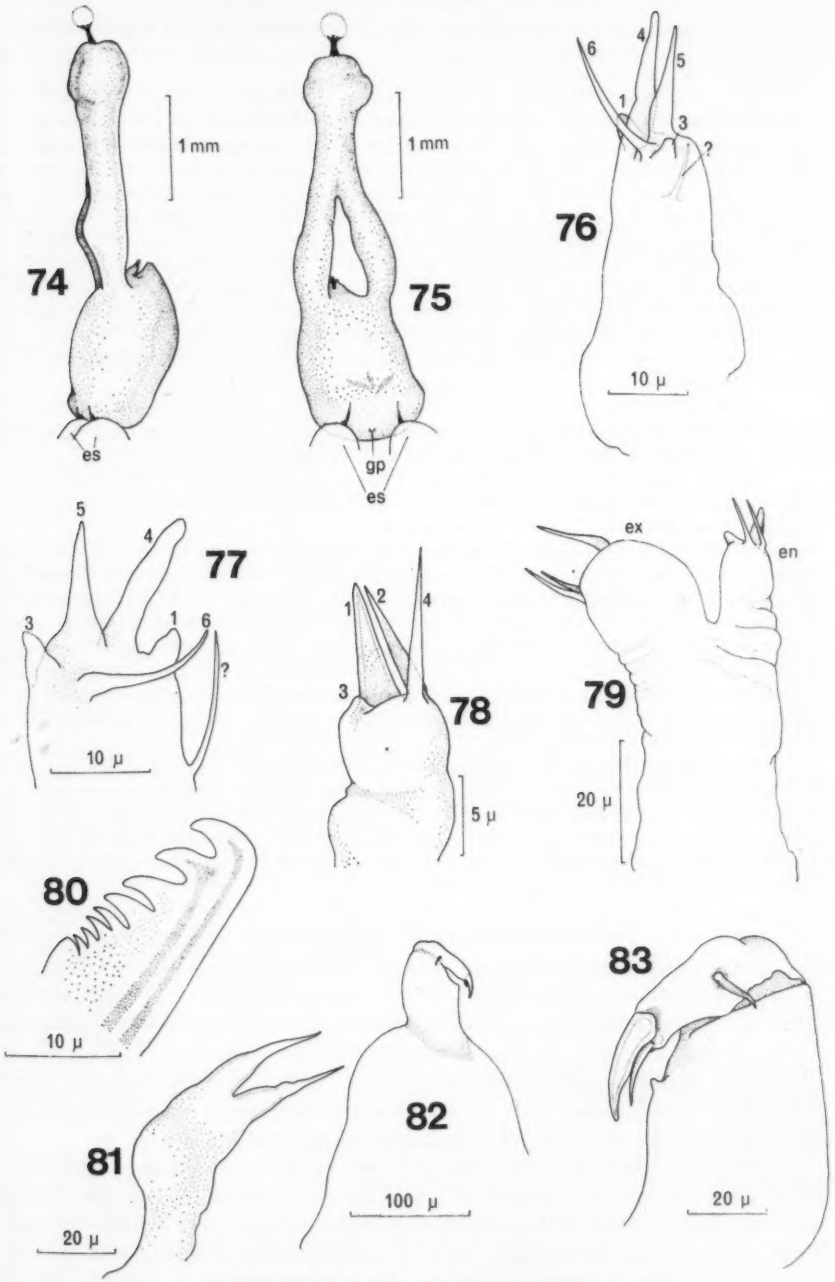
(Fig. 74–83)

This species has never been adequately described, although it has been made a subject of a separate study (Markevich, 1946) and although a separate subfamily has been established for it within Lernaeopodidae. The descriptions so far available fail to bring out the existing homologies between *N. indivisa* and other species of its family.

The description of the female given here is based on specimens found on the gills of *Lepidopsetta bilineata* (Ayres, 1855).

The cephalothorax of *N. indivisa* is greatly reduced. Showing as a mere conical outgrowth on the anterior margin of the trunk (Fig. 74, 75), it is wedged between the bases of the massive second maxillae. The trunk is almost subglobular, slightly longer than wide, the rounded posterior extremity provided with a small genital process (*gp*, Fig. 75), difficult to detect in older specimens.

The first antenna (Fig. 76, 77) is short and appears unsegmented. Its apical armature (Fig. 77) consists of three setae and two tubercles. The setae are the digitiform seta 4, the pointed seta 5 (in this species not bifid), and the long seta 6. Small tubercles 1 and 3 are near the bases of setae 4 and 6, respectively. Tubercle 2 appears to be missing. Another long and slender seta arises from the wall of the antenna some distance from the apex. This seta does not belong to the apical armature (*?*, Fig. 76, 77). It might be homologous with a short seta often found on



FIGURES 74–83. ***Nectobranchia indivisa***.

Fig. 74. Female, entire, lateral; Fig. 75. Same, ventral; Fig. 76. First antenna, entire, dorsal; Fig. 77. Same, tip, lateral; Fig. 78. Second antenna, endopod, ventral; Fig. 79. Same, entire, lateral; Fig. 80. Mandible, tip, lateral; Fig. 81. First maxilla, lateral; Fig. 82. Maxilliped, entire, ventral; Fig. 83. Same, distal part, ventral.

the penultimate part of the first antenna in other Lernaeopodidae (e.g., cf. Fig. 7, 8, and 55 of this paper). On the other hand, its homology might be with the similar long seta found in a similar position on the first antenna of the caligid copepods.

The second antenna (Fig. 78, 79) bears a general resemblance to the same appendage in other Lernaeopodidae, but all traces of segmentation have disappeared and the antenna is often wrinkled and twisted so as to make the recognition of its homologies difficult. It consists of a sympod (Fig. 79), carrying distally the usual two rami. The exopod is bulbous and larger than the endopod. Near its apex there are three long setae, two of them probably homologous with the two papillae found in this place in other Lernaeopodidae. There are no spinules. The endopod (*en*) also appears unsegmented. Its apical armature (Fig. 78), though reduced, still shows many homologies with the other second antennae in the family. The most prominent structure in this armature is a long spine, strong and sharp-pointed, homologous with the hook 1. A more slender seta near its base is the auxiliary seta 2. On the other side of the base is the tubercle 3, difficult to observe in some specimens. In the position occupied by the ventral processes 4 and 5 in most other lernaeopodids, the endopod carries a long seta marked 4 in Fig. 78. No denticulation occurs on the endopod or the sympod of the antenna.

The mandible (Fig. 80) is armed with a continuous series of eight teeth, decreasing in size towards the base of the mandible but not differentiated into the primary and secondary teeth. In this they resemble the most primitive members of the family.

The first maxilla (Fig. 81) is uniramous. There is no trace of the exopod. There are two terminal papillae, surmounted by short but strong setae. There is no seta at the base of the dorsal papilla and no spinulation on the dorsal margin.

The second maxilla (Fig. 74, 75) is fused with its opposite number along the distal halves, leaving between the bases the triangular opening for the cephalothorax. The tips of the appendages are swollen into an almost spherical structure, with the manubrium of the bulla raising from its distal end. The bulla has been described by Gusev (1951) as a six-pointed calyx with a short manubrium and traversed by an extensive system of ducts. Trying to dissect the bulla, I found that the calyx constitutes only a part of the organ of attachment and that a globular, semi-translucent mass fills it and is intimately fused with the host tissue. Attempt at dislodging it ended in its fragmentation and the breaking away of the calyx. It seems sure, however, that it does form a part of the bulla.

The maxilliped (Fig. 82, 83) is unique among Lernaeopodidae. The part of this appendage that would constitute the entire maxilliped in other genera is placed at the tip of a large protuberance, greatly surpassing in size that of the terminal part of the appendage (Fig. 82). The terminal part (Fig. 83) is armed with a small tubercle on its medial margin. The subchela has a long claw and a long auxiliary spine near its base. The seta near the base of the subchela is also unusually long. No denticulation is present.

Comments — The morphology of *N. indivisa* shows a mixture of primitive and advanced features. Among the latter are the second maxillae, the reduction of the cephalothorax unparalleled among Lernaeopodidae, and the peculiar basal part of the maxilliped. Primitive features are the absence of the posterior processes and the lack of secondary teeth in the mandible. The latter, in particular, suggests that *Nectobranchia* is an early offshoot from the main stem of Lernaeopodidae and that it has retained some primitive features while developing some of its characteristic adaptations.

Mention should be made here of the genus *Lernaeopodopsis* Hansen, 1923, with one species, *L. producta*. The second maxillae of that species are also fused along most of their lengths, with the exception of a small triangle between the bases. A similarly reduced cephalothorax fits into this triangle and the structure of the maxilliped is also reminiscent of *N. indivisa*. There

are no posterior processes. Although the description of other appendages is inadequate for comparative purposes, it seems clear that this species is closely related to *N. indivisa* and should be placed in the same genus. I propose, therefore, that *Lernaeopodopsis producta* Hansen, 1923, be renamed *Nectobranchia producta* (Hansen, 1923).

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Primary Production Measurements on a Natural Plankton Bloom[1]

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A comprehensive series of observations made throughout a natural phytoplankton bloom suggested that culture experiments are satisfactory analogues of natural plankton systems, at least under bloom conditions. Calorific values suggested that there was no tendency for the phytoplankton to store fat in the senescent phase of the bloom. No single quantity was found to be a suitable indicator of the physiological vigour of the population.

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INTRODUCTION

THOUGH THE PROCESSES underlying the production of marine phytoplankton can only be elucidated through an integration of knowledge derived from both field observations and culture experiments, the interpretation of the one kind of information in terms of the other is often difficult because culture populations are almost invariably monospecific, whereas natural communities are usually highly diverse. One possible approach to this problem is to make the field observations during phytoplankton blooms, for at these times we expect the species diversity to be at a minimum. Only a few workers have reported repeated measurements during the same bloom (Riley, 1943; Ryther, 1954; Ryther et al., 1958; McAllister et al., 1961; Antia et al., 1963).

The present paper has two main objectives: first, to present and interpret a comprehensive series of data on the development of a marine phytoplankton bloom, and second, to relate the findings to those of culture studies on both local (Nova Scotian) and exotic species. The following quantities were measured: in situ primary production, cell numbers, species diversity, chlorophylls *a* and *c*, particulate carbon, calorific value, inorganic phosphate, nitrate, silicate, temperature, salinity, incident radiation, and water transparency.

MATERIALS AND METHODS

The observations were made in St. Margaret's Bay, Nova Scotia, at station A (64°02′W, 44°35′N) 1 mile from the shore, at 53 m depth. The sampling period extended from March 27

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to April 28, 1969, and the station was occupied 11 times. On each occasion, six depths were sampled: 1, 5, 10, 15, 25, and 40 m.

Primary production was measured by the in situ ^{14}C technique, with incubation lasting from 1000 to 1400 hr local time. Two light bottles and one dark bottle were used at each depth. In addition, at each depth the photosynthesis : respiration (P/R) ratio was estimated by the dissolved oxygen method of Gran (1932). Standing crop of phytoplankton was estimated by several methods. Cell counts were made on sedimented samples preserved in Lugol's solution using the inverted microscope technique. Chlorophyll *a* was determined by the fluorometric method of Yentsch and Menzel (1963). The ratio of chlorophyll *c* to chlorophyll *a* was estimated spectrophotometrically, using trichromatic equations (UNESCO-SCOR, 1966, p. 17). Particulate carbon was measured by the wet combustion method essentially as described in Strickland and Parsons (1965). Species diversity was calculated from the cell counts using the equation

$$D = -\Sigma p_i \log_2 p_i$$

(Margalef, 1968) where D is the diversity and p_i is the probability that any given individual belongs to the ith species. On each sampling day, a sample of phytoplankton for calorific value determination was collected by horizontal towing of a 76-μ mesh net at 5 m. The catch was screened through a 153-μ wire mesh to remove zooplankton and collected on a 76-μ wire mesh. It was rinsed quickly with distilled water and transferred to a plastic bag, which was frozen immediately in dry ice. Later the sample was freeze-dried and the caloric content of the dried material was measured using a Phillipson micro-bomb calorimeter. The ash content was also measured, and the carbon content using the wet-combustion method. Inorganic nutrients were measured on water samples filtered through a 0.45-μ membrane filter. Phosphate was measured by the method of Murphy and Riley (1962), silicate by the method of Grasshoff (1964), and nitrate by the Grasshoff modification of the cadmium amalgum method as described in Strickland and Parsons (1965). Incident radiation was measured by an Eppley 180° pyranometer mounted on the top of the ship's mast. Water transparency was estimated using a two-cell hydrophotometer, with green filters on each cell. The peak sensitivity of this system was 530 mμ with a band width of 70 m. From the data on primary production, calorific value, incident radiation, and water transparency, the biological attenuation coefficient, k_b, which may be used as an index of the efficiency of primary production, was calculated using the following equation (Platt, 1969):

$$k_b = P(z)/I(z)$$

where $P(z)$ is the production at any depth z, and $I(z)$ is the radiation at that depth, both P and I being expressed in calories.

RESULTS

At the beginning of the sampling period, the water column was essentially isothermal at 2.6 C. Salinity increased monotonically with depth from 30.84‰ at the surface to 31.81‰ at 40 m. By April 29 the surface temperature was 5.6 C; the heating was confined almost entirely to the upper 10 m. Salinity at 50 m dropped from 32.24‰ on March 25 to 31.92‰ on April 22.

At the beginning of the study, inorganic nutrients were distributed evenly throughout the water column except for the upper few meters, where values were somewhat lower (Fig. 1). During the bloom, the concentrations of all three nutrients (phosphate, nitrate, and silicate) followed essentially the same pattern. Depletion of nutrients was confined almost entirely to the upper 20 m of the water column. The lowest nutrient levels at the surface were observed on April 16. After that date, the quantity of nutrients in the surface

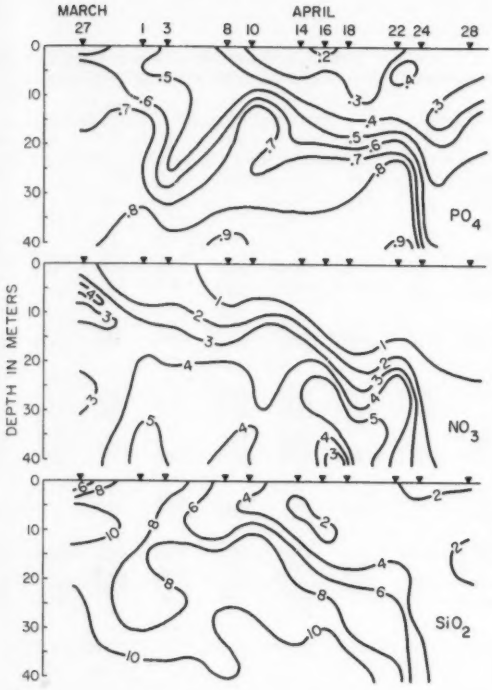


FIG. 1. Concentrations (μg-at/liter) of three inorganic nutrients at station A, St. Maragret's Bay, March 27 to April 28, 1969.

layer gradually increased, until by April 28 the surface values were approaching those that were found before the bloom began. After April 21 there was a sharp decrease in the nutrient levels below 15 m.

Although a total of 31 species were recorded during the study, a minimum of 5 and a maximum of 17 species were present in any individual sample (Table 1). Except *Cylindropyxis* sp., the rest of the taxa have been reported previously from Atlantic coastal waters. *Chaetoceros debile*, *Cylindrotheca closterium*, *Fragilaria* sp., *Gymnodinium* sp., *Navicula* sp., *Rhizosolenia setigera*, *Skeletonema costatum*, *Thalassionema nitzschioides*, *Thalassiosira gravida*, and *Thalassiosira nordenskioldii* were present throughout the sampling period. The species that were most abundant in the bloom were *C. debile*, *T. gravida*, and *T. nordenskioldii*. The dinoflagellate contribution was insignificant.

Cell densities varied from 7.5×10^3 to 7.8×10^6 cells/liter (Fig. 2). The cell numbers increased gradually from March 27, reaching a maximum for the surface on April 10. Below 10 m, numbers continued to increase and reached a maximum on April 16. The maximum cell number at 40 m was attained on April 18. After this, cell numbers decreased sharply throughout the column.

TABLE 1. Numbers of cells per liter recorded at various depths for the m

Phytoplankter	Mar. 27						Apr. 1					
	1 m	5	10	15	25	40	1 m	5	10	15	25	40
Amphidinium sp.	1692	1128	1880	376					377			
Amphora sp.		188		188								
Biddulphia aurita									754			
B. bulchella			564	188								
Ceratium longipes												
Chaetoceros danicum				188								
C. debile	1128	752	1880	4512	752	752	28275	26390	15834	6032	4524	
C. lacinosum							377	377	377			
C. lorenzianum												
C. septentrionale								754				
? *Cylindropyxis* sp.												
Cylindrotheca closterium	5640	5264	9776	8084	4324	3196	11310	7540	13195	5278	5278	1131
Detonula confervacea												
Dinophysis norvegica												
Distephanus speculum			188									
Fragilaria sp.	188	564	752		376	1880	1885	4147	3770	1885	377	
Gymnodinium sp.	7250	2444	14288	10152	3008	1316	5278	6032	1131	3393	754	
Gyrosigma sp.						564						
Licmophora abbreviata	188	188	1128	1128	752		1508					
Navicula sp.	6392	376	2068	4136	3572	1692	7163	2639	1885		1508	1508
Peridinium depressum												
Peridinium sp.												
Pleurosigma aestuarii	376	752	376	188		188			1508			
Prorocentrum micans	188	564	940	940		376	1131	377				
Prorocentrum sp.												
Rhizosolenia setigera			564	188								
Skeletonema costatum	2632	2820	2444	5828			5278	4147	3393	3770	1508	1131
Thalassionema nitzschioides		376	1504			188	6786	2262	4901	3770	754	
Thalassiosira gravida	5452	13160	25944	24440	9776	3760	102544	76908	49010	36569	15457	2639
T. nordenskioldii	2068	3760	3008	3008			3016	377	377		1131	1131
Thalassiothrix frauenfeldii	1504	1128	752				1131					

	Apr. 14						Apr. 16					
	1 m	5	10	15	25	40	1 m	5	10	15	25	40
Amphidinium sp.	2260			565	565							
Amphora sp.								565		7896		1130
Biddulphia aurita				565								
B. bulchella												
Ceratium longipes												
Chaetoceros danicum												
C. debile	5650	6780	21470	230520	5085	3955				31584		
C. lacinosum								565	8475	7896		
C. lorenzianum	2260	565					3390	3390		15792	1695	
C. septentrionale												
? *Cylindropyxis* sp.										7201152		
Cylindrotheca closterium	6215	3955	11865	12430	3955	3955	2260	2260	3390	94752		565
Detonula confervacea												
Dinophysis norvegica												
Distephanus speculum							565					
Fragilaria sp.	5650	6780	10170	6780	565		5650	5650	2260		3390	
Gymnodinium sp.	565	565	2825		565		1695	565		15792		565
Gyrosigma sp.				565								
Licmophora abbreviata	1695	2825		565	565			1130				565
Navicula sp.	5650			4520	3955	2260	2260	2260	1695	7896		1130
Peridinium depressum												
Peridinium sp.							565					
Pleurosigma aestuarii		565								7896		
Prorocentrum micans	565		565	565		565	565			7896		
Prorocentrum sp.		565										
Rhizosolenia setigera		565	565					565		7896		
Skeletonema costatum	6215	8475	19210	24860	5650	3955	2825	2260	7345	15792	2825	565
Thalassionema nitzschioides	3390	4520	18645	1130	4520	3955	5650	5650	7345	23688	3390	1130
Thalassiosira gravida	30510	45200	139555	157070	32770	16950	9605	10170	50850	276360	37855	1186
T. nordenskioldii			1695	1130	565	565	3390	1695	6215	47376	6215	2260
Thalassiothrix frauenfeldii												

toplankton found during the spring bloom of 1969 in St. Margaret's Bay.

Apr. 3				Apr. 8						Apr. 10					
10	15	25	40	1 m	5	10	15	25	40	1 m	5	10	15	25	40
		754		1695	565	565	11865	565			1695	565			
						565		565			1695				
23751	22620	8671	2262	19210	54805	9605	22600	2825	1130	14690	38420	14690	5650	4520	1130
		377													
		377		1130			565	565		1130					
5278	4524	6032	3393	11865	27685	22035	7345	5085	1130	15255	7910	7345	2825	3390	2260
									565	1130					
	377														
1131	3016	2262		3390	5650	5650	3390	2260	2260	6780	5085	6780	6780	3390	3955
4524	2639	4524		565	1130	1130	1130				2260				565
1508	1885	377		1695	3390		1130	1130		2825	1695			565	565
1885	1131	1508	2262	1695	5650	2260	5650	1130	1130	3390	4520	4520	6780	4520	3955
												565			
		377	377	1695	565	2260		1130	565	565		1130		3390	
									1130				565		
6786	3393	3016	1131	15255	14125	2825	5650	1695		15820	2825	5650	2825	565	565
2262				5085	18080	6780		1695	3955	3955	5650	1695	6125	1130	
161356	220545	12818	6032	257075	350865	300015	63280	25425	14125	291540	155375	129950	33900	16950	7910
3770	2639	1508		3390	2260	2260	565			3955	2260	2260	1695	565	
754															

Apr. 18				Apr. 22						Apr. 28					
10	15	25	40	1 m	5	10	15	25	40	1 m	5	10	15	25	40
	565									377	754	377			565
											377				
5085	3955	2825	13560				10170	9605	1130	3770	1508	1131	11865	38420	10170
1130		1130	1130				5085			1131	754				565
		1130									1508	1508	1695		3390
	59890	83055	272895				64975								
1695	2260	2825	2825			565		7345		377	1885	377	2260		3390
														565	
															565
4520	2825	1130	2260	4520	1130	2260	4520	3390	4520	1131	1131	754	2260	14125	4520
			565	1130	3390	2260	5085	3390	2260	754	1508	754	1695	1130	1130
	1695		565												565
565	1695	2825	1130		565	2260	3955			377			1695	2260	2260
											377				
				565	565	565		565							1130
		565	565			565			565						
2260	565	565	1695			1130	4520	565	1695					565	1130
565	565									377					
565									565	754	754			565	
1695	3390	2825	2825				10735	43505	32205	1131	1131		1695	2825	3955
2260	2260		3955	3390	1695	2825	3390	6215	2825	1508		754	3390	3390	5085
26555	90400	122040	19210	7345	6780	25990	27120	41810	25990	1508	3770	1508	5650	10735	7910
565	2825	2260	2825	565	1695	3955	3390	1695	1130			377	1695	1695	565
1695	565	565	565												

The species diversity index varied from 0.57 to 3.30 (Fig. 3). As the bloom developed, the diversity gradually decreased, reaching a surface minimum on April 10. After this, the diversity increased and quickly attained the initial levels. The minimum species diversity observed (0.57 at 15 m on April 16) was associated with large concentrations of *Cylindropyxis* sp.

Chlorophyll *a* varied from 0.38 to 33.83 μg/liter. In the upper 10 m the chlorophyll increased rapidly to a maximum on April 10 (Fig. 4). After April 10, chlorophyll declined rapidly in the upper 10 m, but below 10 m the decline was slower.

Particulate carbon varied from 50 to 872 μg/liter. The changes in carbon (Fig. 5) followed the same pattern as those in chlorophyll *a*.

The *c*/*a* ratio varied from 0.15 to 2.22. At the onset of the bloom, *c*/*a* decreased sharply throughout the column and thereafter increased gradually to a maximum on April 18 (Fig. 6). Initial levels were reached by April 28.

Primary production at the surface varied from 3.24 to 76.37 mg C/m^3 per hr. There was evidence of light inhibition at the surface and on any given day the highest production in the column was usually at 5 or 10 m (Fig. 7). The highest production recorded was 81.65 mg C/m^3 per hr at 5 m on April 10.

The biological extinction coefficient, k_b, varied from 2.3×10^{-4} to 6.0×10^{-2} m^{-1}. At the surface, k_b increased as the bloom developed, reaching a maximum on April 8 (Fig. 8). At successively deeper levels, the peak was reached at progressively later dates, the maximum value being observed at 10 m on April 16. During the rest of the sampling period k_b's declined, the decrease being most pronounced at the surface.

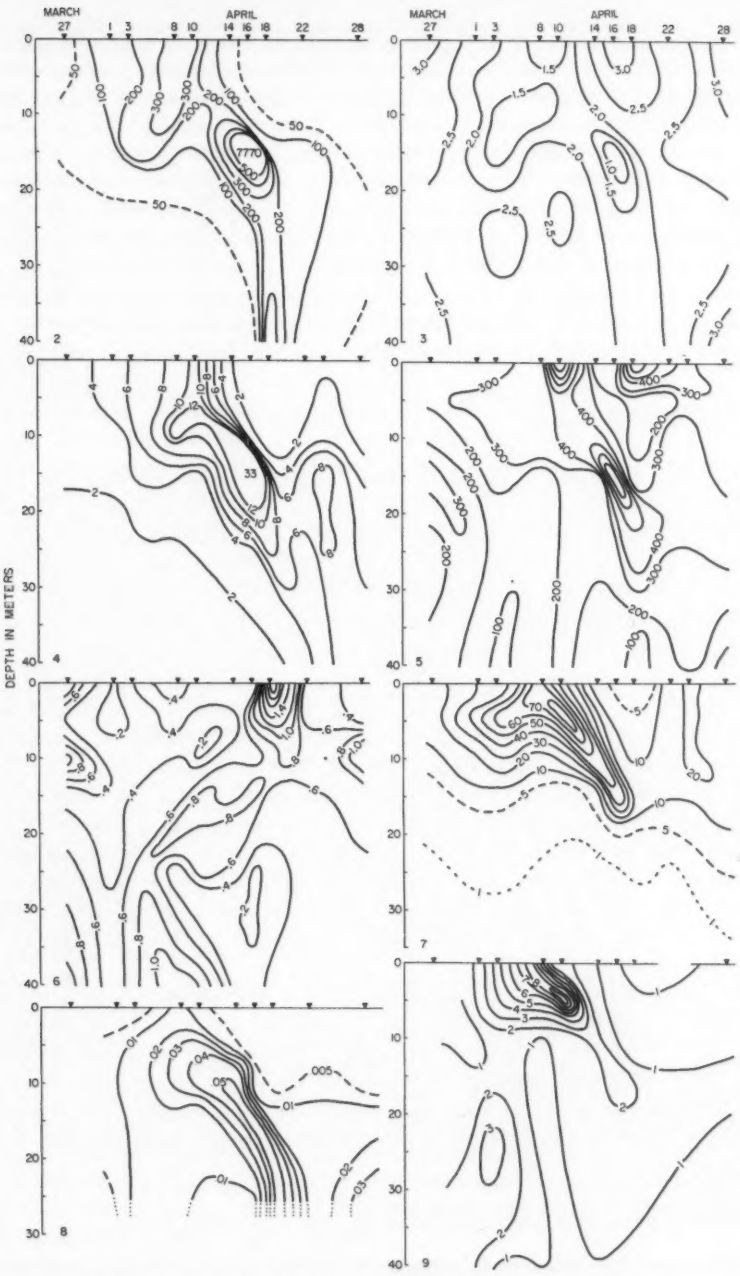
The P/R ratio varied from 0.2 to 9.5 (Fig. 9). In the upper 10 m it increased with the onset of the bloom reaching a maximum at the surface between April 3 and 8, and at 5 m on April 10. Thereafter P/R declined sharply to initial levels. In most of the sampling period P/R was, in general, inversely related to the depth.

Considerable variation was found in both the carbon and caloric contents of the standing stock of phytoplankton. Carbon content varied from 15.0 to 23.0%, and the calorific value varied from 2435 to 4015 cal/g dry wt (Table 2). The caloric equivalent of the carbon fixed in photosynthesis varied between 14.10 and 18.35 kcal/g C. In general, the caloric equivalent of the carbon declined steadily throughout the bloom.

DISCUSSION

Evidence for the Sinking of the Bloom

Though horizontal advection and vertical turbulence may have affected the findings, they were evidently of little importance at least during the period March 27 to April 16. This is reflected in the vertical distribution of salinity and in the evidence given below to demonstrate the sinking of the bloom.



FIGURES 2–9. Other measurements during the spring bloom.
Fig. 2. Phytoplankton cell density (10^3 cells/liter); Fig. 3. Species diversity (bits/cell); Fig. 4. Chlorophyll *a* (mg/m^3); Fig. 5. Particulate carbon (mg C/m^3); Fig. 6. Chlorophyll *c*/*a* ratio; Fig. 7. Carbon-14 uptake (mg C/m^3 per hr); Fig. 8. k_b (m^{-1}); Fig. 9. Photosynthesis/respiration ratio.

TABLE 2. Changes in the ash content, carbon content, calorific value, and caloric equivalent of phytoplankton carbon during the spring bloom of 1969 in St. Margaret's Bay.

Day (Apr.)	% ash[a]	% carbon[a]	kcal/g dry wt	kcal/g C
1	34.8	20.6	3.780	18.360
3	29.8	23.0	4.015	17.500
8	38.1	19.7	2.995	15.175
10	39.5	19.3	3.030	15.690
14	45.0	15.4	2.435	15.800
16	41.7	18.1	2.695	14.900
18	41.6	18.7	2.665	14.270
22	38.8	18.3	2.585	14.105
24	48.7	15.0	2.465	16.475
28	40.6	20.7	3.190	15.445

[a]% of the dried material.

At least two lines of evidence show that the dense and vigorous phytoplankton population, which was present in the upper 10 m layer in the early part of the bloom, sank during the latter part of the bloom and towards the end of the bloom was centered at 25 m. One indication of this is the progressive increase in the total cell numbers below 15 m from March 27 to April 18 (Fig. 2) whereas the primary production throughout this period was both low and nearly constant below 15 m. The same trend is apparent in chlorophyll *a* (Fig. 4) and particulate carbon (Fig. 5). The same phenomenon may be seen even more clearly by considering the changing distribution with depth of some of the individual species such as *Chaetoceros debile*, *Cylindrotheca closterium*, *Skeketonema costatum*, *Thalassiosira gravida*, and *T. nordenskioldii* (Table 1).

The sudden appearance of *Cylindropyxis* sp. (Table 1) in large concentrations on April 16 indicates the influence of some lateral water movement, but this does not invalidate the remarks made in the previous paragraph. The abrupt disappearance of this species on April 22 also suggests advective process, this time also associated with some vertical mixing, since a sharp decrease in nutrients and a reduction in 50 m salinity from 32.2 to 31.9‰ were also observed.

As with laboratory cultures (Fogg, 1965) the present bloom may be considered in the following phases: the *exponential phase* occupied the period up to April 8 at the surface. At this point, the beginning of the *stationary phase*, the population, although still productive, began to sink. By April 14, the minimum cell densities had been reached at the surface. We designate this as the onset of the *senescent phase*, which persisted until the end of the study.

NUTRIENT DEPLETION AND CHANGES IN THE STANDING CROP

Because of the suspected influence of lateral advection discussed above, the following remarks on utilization rates and production are largely confined

to the period prior to April 16. The relations between each of the inorganic nutrients in the water column and the age of the bloom were studied by linear regression. The slopes of the regression lines gave the average utilization rates of the nutrients. These rates were 15.3 mg-at/m^2 per day for silicate, 6.4 mg-at/m^2 per day for nitrate, and 0.47 mg-at/m^2 per day for phosphate. During the same period, carbon accumulated at a net rate of 4.8 mg-at/m^2 per day and chlorophyll *a* at 7.2 mg/m^2 per day. The ratio of the change in nitrate to that in phosphate, $\Delta N:\Delta P$, was 13.5, which compares favourably with the figure of 15 given by Redfield et al. (1963). The relative change in silicate, $\Delta Si:\Delta P$, was 32.6 compared with a ratio of 16 suggested by the same authors. Redfield's figure was an average value for the phytoplankton of the world oceans. A higher ratio obtained on a bloom consisting entirely of diatoms is not unexpected.

The relative net change in carbon, $\Delta C:\Delta P$, was 10.2 when calculated from particulate carbon data. This is an order of magnitude different from the value of 106 suggested by Redfield et al. (1963). However, the present method of calculation ignores those fractions of assimilated carbon that are subjected to respiration, grazing, or excretion. Judging by the very small standing stock of zooplankton observed during the day, the grazing effect could be taken as negligible. However, the unpublished observations of Dr D. D. Sameoto of the Marine Ecology Laboratory indicate that in St. Margaret's Bay there is a large population of zooplankton that live close to the bottom during the day, and therefore are difficult to sample, but migrate to the surface layers during the night; these animals could exert considerable nocturnal grazing pressure. In the photic zone, respiration varied from 50 to 10% of the photosynthesis through the bloom, and. therefore could not by itself account for the low $\Delta C:\Delta P$ ratio. The amount of fixed carbon lost by grazing, respiration, and excretion may be estimated by taking the production rate for a given sampling day, estimating the increase in carbon on the next sampling day, and comparing this with the change actually observed in particulate carbon. This calculation gave an average loss rate of 60% of the fixed carbon. The data are insufficient for this figure to be portioned among grazing, respiration, and excretion, but it seems likely that excretion of some carbon (*unlabelled* with ^{14}C) did occur. Antia et al. (1963) sought to explain a discrepancy between production estimates from ^{14}C and from oxygen techniques by the excretion of *labelled* carbon. But there is no real contradiction, since in the experiments of Antia et al. the incubation time was 24 hr, which would allow ample time for the recycling of labelled material, whereas in our experiments the incubation time was 4 hr, which is thought to be a sufficiently short time that excretion of newly labelled carbon could not occur (Strickland, 1960).

If the ratio $\Delta C:\Delta P$ is now calculated from the ^{14}C data rather than the particulate carbon data, a value of 80 is obtained, which is in better agreement with Redfield's (1963) figure of 106.

On the basis of the particulate carbon and chlorophyll results the following linear regression equation was constructed:

$$\text{carbon} = 215 + 16.0 \times \text{chl } a$$

The equation is based on 71 pairs of data and the correlation coefficient is highly significant ($r = 0.55$, $P < 0.01$). This equation may be useful in estimating the carbon content of the phytoplankton standing crop when the chlorophyll concentration is known. Since it is well known that there is considerable variation in the carbon:chlorophyll relation in both time (Steele and Baird, 1961, 1962) and space (Lorenzen, 1968) this equation would be of most use when applied to bloom conditions in St. Margaret's Bay.

Indices of Physiological State

In this section, three potential indices of the physiological state or vigour of phytoplankton communities are considered. These are the calorific value of the plankton, the ratio of chlorophyll *c* to *a*, and the ratio of photosynthesis to respiration.

At every depth, the ratio of chlorophyll *c* to *a* increased at the onset of the bloom and decreased thereafter (Fig. 6). Above 10 m the ratio began to decrease on April 18, i.e., during the senescent phase. Below 10 m, the decrease began somewhat earlier. This pattern of variation in the *c*/*a* ratio with age of the population is apparent in the culture experiments of Humphrey (1963) and Madgwick (1966), the plastic bag experiments of McAllister et al. (1961) and Antia et al. (1963), and culture experiments of Subba Rao (unpublished data) in this laboratory. But although the pattern of variation of *c*/*a* with age may be consistent from population to population, its usefulness as an indicator of either the age of a bloom or its physiological state may be limited by the ambiguity arising from the fact that the parabolic nature of the variation implies that there will be two possible values of age (or vigour) for every value of the *c*/*a* encountered.

The value of the photosynthesis: respiration ratio, P/R, also went through a maximum at each depth, but the maxima did not occur at the same times as those for *c*/*a*. At the surface, the maximum value of P/R was observed on April 8, and at greater depths in the euphotic zone the maxima occurred at successively later dates (Fig. 9). We believe that this shift in the maximum is associated with the sinking of the bloom as discussed above. The pattern of variation of P/R with age of the population (Fig. 6) differs from the results obtained in cultures by Ryther (1956) and Humphrey and Subba Rao (1967) and in the experiments of Subba Rao (unpublished data) in this laboratory, who found a monotonic decrease of P/R with age. However, the present data are consistent with the observation made by Subba Roa (1969) on a natural bloom of *Asterionella japonica*. Thus, in natural populations at least, the value of P/R as an indicator of age (or vigour) of a bloom may be subject to the same limitations as those mentioned above for *c*/*a*.

Measurements of the calorific value of the phytoplankton population were made at 5 m only. The results are, to the best of our knowledge, the first published data on variations in the calorific content of a natural marine

phytoplankton population. The decrease in the caloric equivalent of the phytoplankton carbon as the bloom progressed (Table 2) confirms the results of the plastic bag experiment reported by Antia et al. that there was no tendency to store fat in the ageing population. If there had been a significant increase in the fat content of the cells, there would have been an observable increase in the calorific value, because fat has the highest calorific value of all the major constituents of the organic matter of plankton (Ostapenya and Sergeev, 1963).

The calorific value of the phytoplankton might serve as an index of physiological vigour if the sampling problem could be overcome. It is time-consuming to collect the samples and it is difficult to take samples that are uncontaminated by zooplankton. We were fortunate that there was little zooplankton present in St. Margaret's Bay during this study. The purity of the samples was checked by suspending some of the freeze-dried material in cedar oil and observing in the microscope. The samples were free from contamination by zooplankton. We feel that there is a need for much more systematic data on the calorific values of both natural and culture populations of phytoplankton.

Efficiency of Primary Production

An idea of relative efficiency of primary production may be gained by calculation of the quantity k_b as defined by Platt (1969). This parameter estimates that proportion of the absorption of submarine light that is caused by photosynthesis. The magnitude of k_b increased with depth and the peak at successively deeper depths occurred at progressively later dates (Fig. 8). This latter aspect was associated with the sinking of the population described above; k_b tends to be higher when the biomass is higher.

The regression of k_b on chlorophyll *a* was calculated for each depth. The regression was highly significant in each case. The slopes of the regression lines at 1 and 5 m were different ($t = 5.81$, 18 df, $P < 0.01$) but not those at 5 and 10 m ($t = 0.7$). The slope of the 15-m regression was greater than that for 10 m ($t = 5.35$, $P < 0.01$) and that for 25 m greater than that for 15 m ($t = 3.47$, $P < 0.01$). Comparison of these regression lines with those calculated by Platt (1969) for the same station from observations made during the period May to November 1968 showed no significant difference between the two sets of regression lines for any of the depths considered. However, values of k_b were considerably higher, by roughly an order of magnitude, during the bloom than at other times, on account of the greater standing stock present.

Species Diversity

Values of species diversity (Fig. 3) fall within the range indicated by Margalef (1968) for coastal waters. The pattern of variation of species diversity was similar to that of the chlorophyll *c*/*a* ratio (Fig. 6). In this respect, the *c*/*a* ratio is analagous to the pigment index (i.e., ratio of optical densities at 430 and 665 mμ of acetone extract of plankton) of Margalef (1968), which has been found to be a relatively good predictor of species diversity.

CONCLUSIONS

The primary conclusion from this work is that in most respects culture experiments are satisfactory analogues of natural plankton systems, at least under bloom conditions. Close correspondence was shown between the variation in a spring bloom and in culture experiments for chlorophyll c/a and P/R. Values of the assimilation number (mg C fixed/hr per mg chl a) encountered in the top 10 m during the present study ranged from 1.0 to 12.3 and were comparable to those found in cultures of diatoms isolated from local waters by Subba Rao (unpublished data), or those summarized by Yentsch and Lee (1966). Similarly the magnitudes of the index k_b measured in the bloom were of the same order as those observed on cultures.

Some points of divergence between the two approaches include the artificially large values of biomass and nutrient concentration usually employed in culture studies. These have been partially rectified in the work of Strickland's group, using large plastic spheres. This approach suffers from the drawback that vertical structure in the system is not allowed to develop. The recent work in a deep tank (Strickland et al., 1969) will obviate some of these difficulties. An unresolved question is the variation in the calorific value with the age of a population. Work on a natural bloom showed a steady decline with age whereas observations on the fat content of diatom cultures (Lewin and Guillard, 1963) would suggest that calorific value might increase in the senescent phase because of a tendency to store fat. Another wide difference between culture studies and field studies arises because culture work has been confined almost entirely to monospecific ("pure") populations; under these circumstances there is no possibility of the development of feedback interactions associated with high species diversity. Further, even at the peak of the bloom in the present study, the population could not be described as monospecific; there were never less than five species in any one sample. There is consequently a pressing need for systematic laboratory studies of mixed populations.

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Parasites of the Threespine Stickleback (*Gasterosteus aculeatus*) in Newfoundland and Labrador[1]

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HANEK, G., AND W. THRELFALL. 1970. Parasites of the threespine stickleback (*Gasterosteus aculeatus*) in Newfoundland and Labrador. J. Fish. Res. Bd. Canada 27: 901–907.

Fifteen genera of helminths (19 species) and two genera of parasitic copepods (2 species) were recovered from 375 threespine sticklebacks (*Gasterosteus aculeatus* Linnaeus, 1758) taken in 10 sampling areas in Newfoundland and Labrador during 1968 and 1969. Two new host records and two new records for North America are included.

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INTRODUCTION

THE THREESPINE STICKLEBACK, *Gasterosteus aculeatus* L., a euryhaline fish that is common in the Holarctic (Sterba, 1963; Leim and Scott, 1966; Wheeler, 1969), is host to many species of parasites throughout the whole of its range. Many of the relevant records were brought together by Hoffman (1967).

Since that time a number of papers have dealt with parasites of this species of fish (Arme and Owen, 1967; Chappell, 1969a, b, c; Chappell and Owen, 1969; Hanek and Threlfall, 1969a, b, c, 1970; Threlfall, 1968; Vik et al., 1969). The parasites of the fish of Newfoundland and Labrador are relatively poorly known; therefore, a survey of the threespine stickleback was initiated in November 1968 to determine what parasites, if any, they harbour in this province.

MATERIALS AND METHODS

Three hundred and seventy-five threespine sticklebacks were caught at 10 localities in Nfld. and Labrador during the period November 1968–September 1969 (Table 1, Fig. 1). The fish were caught with a 30-ft hand seine, and transported alive to the laboratory or field station, where they were examined, by conventional parasitological techniques, for helminth and copepod parasites.

Monogenetic trematodes were fixed in a mixture of glycerol and ammonium picrate (Malmberg, 1956) and examined by phase contrast microscope. Digenetic trematodes and cestodes were fixed and stored in 5% formalin and afterwards stained with Semichon's aceto-carmine and mounted in Canada balsam. Nematodes were fixed and stored in 70% ethanol and were later cleared in lactophenol and mounted in glycerine jelly. Trichodinids were sometimes seen, the number of fish infested being noted.

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TABLE 1. Origin and number of *Gasterosteus aculeatus* examined and number and percentage (in parentheses) infected with helminths and parasitic copepods.

No.	Locality	Date	No. examined	No. infected
1.	Witless Bay** [a]	Nov. 22, 1968	25	21(84)
		Jan. 23, 1969	8	6(75)
		Feb. 19, 1969	25	25(100)
		Mar. 31, 1969	20	9(45)
		Apr. 30, 1969	25	22(88)
		May 27, 1969	25	25(100)
		June 20, 1969	25	25(100)
	Total:		153	133(86)
2.	St. John's area			
	a. Long Pond* [a]	Mar. 22, 1969	15	15(100)
	b. Quidi Vidi**	Jan. 17, 1969	10	9(90)
	c. Middle Cove*** [a]	June 25, 1969	10	10(100)
	Total:		35	34(97)
3.	Conception Bay			
	a. Manuels R.*	May 21, 1969	25	25(100)
	b. Portugal Cove***	May 28, 1969	7	7(100)
	Total:		32	32(100)
4.	Ocean Pond*	May 30, 1969	10	10(100)
5.	Broad L. Bellevue**	Dec. 14, 1968	25	21(84)
		June 27, 1969	10	10(100)
	Total:		35	31(88)
6.	St. Mary's Bay			
	a. Mitchells Br.**	June 17, 1969	15	15(100)
	b. Pond near Colinet*	June 4, 1969	15	15(100)
	Total:		30	30(100)
7.	Mary's Hbr. — Labrador			
	a. Mary's R.*	July 10, 1969	15	9(60)
	b. Mary's Hbr.**	July 16, 1969	10	10(100)
	Total:		25	19(76)
8.	Eagle R.*	July 26, 1969	20	19(95)
9.	Grand L. and vicinity			
	a. Grand L.*	Sept. 1, 1969	10	10(100)
	b. Caribou R.*	Sept. 7, 1969	5	5(100)
	c. Naskaupi R.*	Sept. 7, 1969	5	5(100)
	Total:		20	20(100)
10.	Nain Hbr.**	Aug. 10, 1969	15	12(80)
	Total		375	340(90)

[a] * = fresh water; ** = brackish water; *** = salt water.

RESULTS AND DISCUSSION

Three hundred and forty fish (90%) were infected with helminth and copepod parasites, the percentage incidence being similar in fish from each of the three major habitat types sampled (freshwater, brackish, saltwater). Table 2 details the parasites found in the various sampling areas. The most

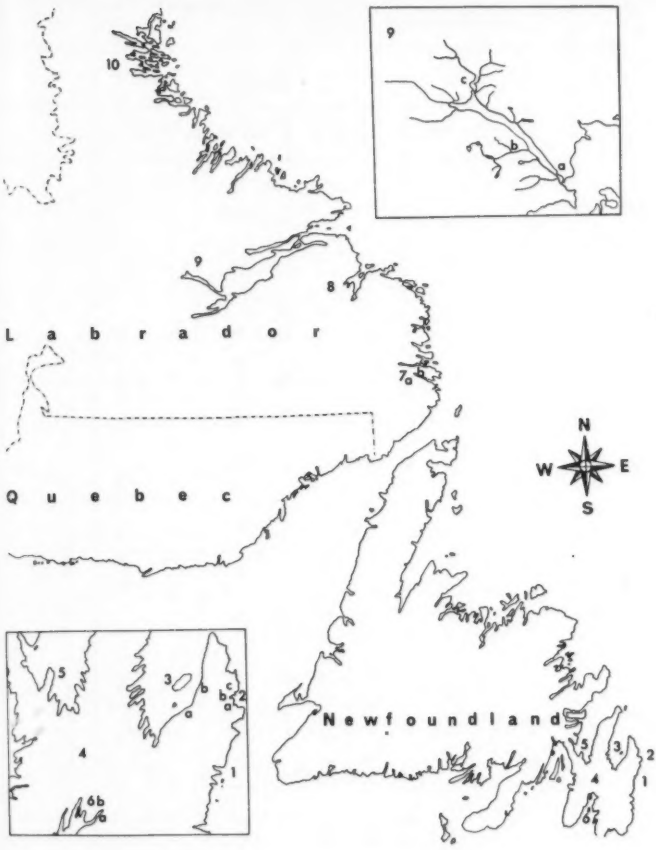

FIG. 1. Location of areas sampled.

commonly found and widely distributed parasite was *Gyrodactylus avalonia* this species being found in all the areas sampled, except Mary's River (*7a*).

Thirteen species of parasites were recovered from fish caught at Witless Bay (Table 3), a higher number than in any other area. The next highest number of species from one area was nine from Ocean Pond. The large number of species found in fish from Witless Bay may be because this region was sampled over a period of 7 months, some parasite species being found in one month and not the next. This demonstrates the need for sampling a population of a particular host over a period of time to obtain an accurate picture of its parasite burden, and why one sample from an area might be misleading. Apparently *Gyrodactylus memorialis* and *Derogenes varicus* are seasonal in their appearance (Table 3). However, more work will have to be done to determine whether this was a true picture of the infection pattern, or whether it was due to variations in sampling techniques or other factors.

TABLE 2. Infection of *Gasterosteus aculeatus* with helminths and copepods. See Table 1 for identification of locality.

Species	Locality																
	1	2*a*	2*b*	2*c*	3*a*	3*b*	4	5	6*a*	6*b*	7*a*	7*b*	8	9*a*	9*b*	9*c*	10
Gyrodactylus avalonia Hanek and Threlfall, 1969	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
G. canadensis Hanek and Threlfall, 1969	+	+	−	+	+	+	+	+	+	+	−	−	−	−	−	−	−
G. lairdi Hanek and Threlfall, 1969	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
G. memorialis Hanek and Threlfall, 1969	+	−	+	−	+	+	−	+	−	−	−	−	−	−	−	−	−
Lecithaster gibbosus (Rud., 1802)	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
Brachyphallus crenatus (Rud., 1802)	+	−	+	+	+	+	−	+	−	−	−	+	−	−	−	−	+
Derogenes varicus (Mueller, 1784)	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Crepidostomum cooperi Hopkins, 1931	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
*Bunodera luciopercae** a (Müller, 1776)	−	−	−	−	−	−	+	−	+	−	−	+	−	−	−	−	−
Podocotyle atomon (Rud., 1802)	+	−	−	+	+	−	−	+	−	+	+	+	−	−	−	−	−
*Diplostomulum spathaceum*** a (Rud., 1819)	+	−	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−
*Proteocephalus filicollis*** (Rud., 1802)	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
Proteocephalus sp.	−	−	−	−	−	+	+	−	−	+	+	−	+	+	+	+	−
Bothriocephalus sp.	+	−	+	−	+	−	−	+	−	−	−	−	−	−	−	−	−
Schistocephalus solidus (Müller, 1776)	−	−	−	−	−	−	+	−	−	−	−	−	−	+	+	+	−
*Ascarophis morrhuae** Beneden, 1871	+	−	−	−	−	+	−	+	+	+	+	+	+	+	−	−	+
Contracaecum sp.	−	−	−	−	+	−	−	−	−	−	−	−	+	+	−	−	−
Neoechinorhynchus rutili (Müller, 1780)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Acanthocephalus lateralis (Leidy, 1851)	+	−	−	+	−	+	+	−	+	−	+	+	−	−	−	−	−
Ergasilus auritus Markewitsch, 1940	−	−	−	−	−	−	+	−	−	+	−	+	−	+	+	+	−
Thersitina gasterostei (Pagenstecher, 1861)	+	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	+

a* = new host record; ** = new record for North America.

The average number of species per sampling area in insular Newfoundland was higher than for the Labrador areas (7.0:5.1). The total number of parasite species recovered from insular Newfoundland was greater than from Labrador (20:13), this difference perhaps being explicable in terms of physicochemical differences in the host's environment in the two regions.

TABLE 3. Infection of *Gasterosteus aculeatus* collected at Witless Bay.

Species	Nov. 22, 1968			Jan. 23, 1969			Feb. 19, 1969			Mar. 31, 1969			Apr. 30, 1969			May 27, 1969			June 29, 1969		
	a[a]	*b*[a]	*c*[a]	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*
Gyrodactylus avalonia Hanek and Threlfall, 1969	60	1–7	2.2	37	1–2	1.3	76	1–15	2.9	20	1–2	1.5	84	2–20	6.1	100	2–20	10.0	96	5–42	13.0
G. canadensis Hanek and Threlfall, 1969	–	–	–	–	–	–	8	1–3	2.0	–	–	–	8	1	1.0	24	1–4	2.4	32	1–5	3.0
G. lairdi Hanek and Threlfall, 1969	–	–	–	25	1–3	2.0	12	1–2	1.3	4	2	2.0	4	1	1.0	12	1–2	1.7	16	1–2	1.7
G. memorialis Hanek and Threlfall, 1969	–	–	–	4	1–3	2.0	8	1–2	1.5	4	1	1.0	8	1–3	2.0	–	–	–	–	–	–
Lecithaster gibbosus (Rud., 1802)	–	–	–	15	1–3	2.0	4	1	1.0	5	1	1.0	12	1–2	1.5	12	2–3	2.0	–	–	–
Brachyphallus crenatus (Rud., 1802)	12	1	1.0	8	2–4	3.0	24	1–2	1.0	10	1	1.0	8	1	1.0	28	1–4	2.2	–	–	–
Derogenes varicus (Mueller, 1784)	–	–	–	–	–	–	4	1	1.0	5	1	1.0	–	–	–	–	–	–	–	–	–
Podocotyle atomon (Rud., 1802)	28	1–12	3.2	–	–	–	32	1–4	2.5	4	1	1.0	8	1–2	1.5	12	1–5	2.5	20	1–3	1.5
Diplostomulum spathaceum (Rud., 1819)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8	2–8	4.0	20	6–35	13.0
Bothriocephalus sp.	4	2	2.0	–	–	–	10	1–4	2.5	4	2	2.0	8	1–3	2.0	–	–	–	–	–	–
Ascarophis morrhuae Beneden, 1871	4	1	1.0	12	1	1.0	48	1–5	2.1	8	1	1.0	20	1–8	3.2	4	1	1.0	8	1–2	1.5
Acanthocephalus lateralis (Leidy, 1851)	–	–	–	–	–	–	4	8	8.0	–	–	–	4	2	2.0	20	1–3	1.8	4	1	1.0
Thersitina gasterostei (Pagenstecher, 1861)	–	–	–	4	3	3.0	4	2	2.0	4	2	2.0	4	1	1.0	8	1–2	1.5	8	1–4	2.5

[a] *a* = incidence, percentage; *b* = intensity, range; *c* = intensity, average.

Shulman and Shulman-Albova (1953), working on the parasites of fish from the White Sea, recorded 15 species of helminths and one species of parasitic copepod (*Thersitina gasterostei*) from threespine sticklebacks taken at two sampling stations (Cernorecenskaja Bay; Gridinskaja Bay) in the Kandalaksha Gulf and at two stations (Virma Bay; 2.5 km upstream from the mouth of the Virma River) in the Gulf of Onega. The distribution of parasites in the areas sampled was not homogeneous, some species being present in one area, absent in another. The same situation was seen in the present work (Table 2).

Bunodera luciopercae, a digenean that has been recovered from *Perca flavescens* (Mitchill) and *Micropterus salmoides* (Lacépède) in North America (*fide* Hoffman, 1967) and that is common in many fish genera in Europe, was recovered from the threespine stickleback for the first time.

Although *Diplostomulum spathaceum* has been recovered from a wide variety of hosts in North America, it has never before been recorded from the threespine stickleback. Shulman and Shulman-Albova (1953) noted that this species was present in 6.6% of the sticklebacks that they examined from Cernorecenskaja Bay.

Proteocephalus fillicollis has been recovered from several species of coregonids in North America (Benedict, 1900), and from *Eucalia inconstans* (Kirtland (*fide* Hoffman, 1967). It is an extremely common parasite of *Gasterosteus* spp. in Europe (Hopkins, 1959; Bykhovsky et al., 1962; Chappell and Owen, 1969), but has not previously been recovered from *G. aculeatus* in North America.

The genus *Ascarophis* Van Beneden, 1870, was reviewed by Dollfus and Campana-Rouget (1956), who described a new species (*Ascarophis crassicollis*) from the bib or pouting (*Gadus luscus* L.). *Ascarophis morrhuae* has been described from several species of marine fish taken in European waters. The present record is a new host record, the measurements of the specimens falling within the range given by Dollfus and Campana-Rouget (1956).

On many occasions trichodinids were seen on the body surface and gills of the fish examined. A record was kept of the number of fish infected, although no specific identification of the trichodinids was attempted. Lom and Laird (1969) reported that threespine sticklebacks caught in brackish water at Witless Bay, Nfld., were often heavily infected with *Trichodina tenuidens* Fauré-Fremiet, 1943. The following list shows: (*a*) the locality where fish were obtained; (*b*) the month they were captured; and (*c*) the percentage infected with *Trichodina* sp.: 1, May, 8; 1, June, 4; 2*a*, March, 20; 2*b*, January, 30; 3*a*, May, 100; 3*b*, May, 100; 4, May, 100; 5, December, 100; 5, June, 100.

If no reference is made to a certain locality or date, no *Trichodina* sp. were found on fish from that area, or on that particular date. No fish from Labrador were infected with these protozoans.

ACKNOWLEDGMENTS

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Stress of Anesthesia with M.S. 222 and Benzocaine in Rainbow Trout (*Salmo gairdneri*)

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Rainbow trout (*Salmo gairdneri*) anesthetized with M.S. 222 for periods up to 12 min experience interrenal ascorbate depletion, uremia, and moderate hypercholesterolemia. Anesthesia with neutralized M.S. 222 (*p*H 7) or benzocaine prevented these changes and significantly reduced the variability in plasma glucose, cholesterol, and cortisol, indicating that the stress of anesthesia with M.S. 222 is due to the low *p*K of the sulfonic acid moiety.

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INTRODUCTION

MAMMALS ANESTHETIZED WITH CERTAIN COMPOUNDS (e.g., diethyl ether) experience a marked stress reaction involving activation of the pituitary–adrenal axis, whereas anesthesia with other compounds, notably the barbiturates, does not cause this effect (Moore, 1957; Hedner and Rerup, 1962). The resulting metabolic changes include hypersecretion of 17-hydroxycorticosteroids accompanied by corresponding changes in blood chemistry together with a marked adrenal ascorbic acid depletion (Sayers, 1957).

Little is known of the stress response of fishes to anesthesia, though anesthetics are widely employed both in research and in hatchery practice (Schoettger et al., 1967). Preliminary work has indicated that M.S. 222 (ethyl *m*-aminobenzoate methanesulfonate), a commonly used anesthetic, can cause pituitary activation in rainbow trout (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*) (Wedemeyer, 1969). However, Black and Connor (1964) found no changes in blood and muscle lactate, hemoglobin, or muscle glycogen in rapidly anesthetized rainbow trout. On the other hand, Randal (1962) showed that M.S. 222 anesthesia at a slower rate increases the heart and respiration rate of teleost fishes, probably via the parasympathetic innervation. There are thus conflicting inferences in the literature on possible biochemical changes in fish anesthetized with M.S. 222.

With this background in mind, a series of experiments was designed to evaluate the "stressor" properties of M.S. 222 anesthesia. Rainbow trout were selected as the test species and their stress response was evaluated, using interrenal ascorbate depletion and plasma cortisol levels as a measure of pituitary activation (adrenocorticotrophin production). Plasma cholesterol

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and glucose were measured to evaluate liver function, and plasma chloride and blood urea nitrogen (BUN) to evaluate renal and gill function. These results were compared to those from fish anesthetized with neutralized M.S. 222 (*p*H 7), or benzocaine (ethyl *p*-aminobenzoate), a close chemical relative that shows considerable promise as a fish anesthetic (McErlean and Kennedy, 1968).

METHODS

Groups of 10 yearling rainbow trout held at 10 C in water of 20 ppm hardness ($CaCO_3$) were anesthetized with sufficient M.S. 222 or benzocaine to cause loss of the righting reflex in 3–5 min. This required 80 ppm (0.03 mM) of M.S. 222 or neutralized M.S. 222 and 50 ppm (0.03 mM) of benzocaine. The M.S. 222 was either adjusted to *p*H 7 with 0.1 N NaOH before use or used in its sulfonic acid form. Neutralized M.S. 222 (free base form) was as efficient an anesthetic as the sulfonate.

The levels of anesthesia chosen did not completely abolish opercular movement during a 12-min period, medullary collapse did not occur, and the fish could be revived by flushing the gills with fresh water. After immobilization, the fish were removed serially at about 1-min intervals and blood and anterior kidney samples were taken for analysis. The time exposed to the anesthetic thus ranged from about 3 min (for the first fish removed) to 12 min (for the last fish removed). After taking blood from the severed caudal peduncle, each fish was iced and the anterior kidney removed as quickly as possible. All experiments were done in triplicate but diurnal variations in glucocorticoids were neglected (Zimmerman and Critchlow, 1967).

Interrenal ascorbic acid analyses were performed by the method of Polk et al. (1970) as modified for interrenal tissue by Wedemeyer (1969). Plasma glucose analysis was by the *o*-toluidine method of Dubowski (1962); cholesterol by the method of Searcy et al. (1962); cortisol by the method of Smith and Muehlbaecher (1969); BUN by the method of Chaney and Marbach (1962); and chloride by standard chloridometer techniques.

Statistical analysis of the data was done on an IBM 1130 digital computer. Regression lines, T tests for zero slope, correlation coefficients, and F tests of variance ratios were calculated. Regression lines calculated from individual replicates were not significantly different from each other and so were pooled for a better estimate of the slope.

RESULTS AND DISCUSSION

Interrenal ascorbate levels are presented in Fig. 1 as a function of exposure time to either M.S. 222, neutralized M.S. 222, or benzocaine. Of the three anesthetics, only the slope of the M.S. 222 regression line is statistically different from zero ($T > 2.3$, $P = 0.05$), indicating that anesthesia with this compound is associated with a progressive interrenal vitamin C depletion. The correlation coefficient was −0.7. Since ascorbate depletion is an indirect measure of ACTH (adrenocorticotrophin) production, these data imply that under the conditions employed, anesthesia with M.S. 222 is a physiological stress that results in activation of the pituitary–interrenal axis. The fact that adjusting the M.S. 222 to *p*H 7 before use eliminated ascorbate depletion (Fig. 1) implies that the sulfonic acid was probably responsible for the stress effect. Further evidence for this is the fact that in the water supply used, 80 ppm M.S. 222 solutions are quite acid (*p*H 4) and apparently cause substantial gill irritation as shown by the agitated behavior of fish when they contact the anesthetic. This initial stimulation of agitated swimming, which

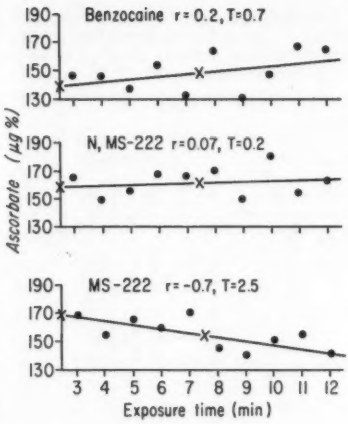

FIG. 1. Interrenal ascorbate levels in rainbow trout as a function of time exposed to 0.03 mM benzocaine, neutralized M.S. 222, or M.S. 222, at 10 C. Correlation coefficient (r) and t-test for zero slope (T) are given.

has been noted by previous workers (Black and Connor, 1964), does not occur when the anesthetic is used at pH 7. Since the pK of M.S. 222 is about 3.5, it is virtually all in the free base form (ethyl m-aminobenzoate) at pH 7, although this does not noticeably affect its efficacy as an anesthetic. That this is true, even though the water-solubilizing effect of the sulfonic acid moiety is removed, can probably be accounted for by the increased lipid solubility, and hence gill transport, of the free base.

Benzocaine (ethyl p-aminobenzoate) is clearly similar structurally to neutralized M.S. 222 and its stress effect (interrenal ascorbate depletion) was predictably mild (Fig. 1). Although there was a slight upward trend in the ascorbate regression line, it was not statistically significant ($T < 2.3$). Thus, vitamin C analyses following benzocaine or neutralized M.S. 222 anesthesia would give unbiased results, whereas M.S. 222 would tend to give low results if the anesthesia were prolonged more than a few minutes, as it would be if several fish were anesthetized together.

For plasma cortisol levels (Table 1), the slopes of the calculated regression lines revealed no statistically significant trends in the data ($T < 2.3$). Since the total period of exposure to the anesthetics was limited to about 12 min, there probably was not sufficient time for cortisol synthesis to become significant. Previous work with a related salmonid at a similar temperature has shown that a noticeable cortisol response to stress does not occur until about 20 min after exposure to the stressor (Wedemeyer, 1969). Thus, although ACTH production as measured by interrenal vitamin C depletion occurs immediately, the glucocorticoid (stress) hormone response is not as quickly evident. Similar results have been reported in mammals exposed to the stress of diethyl ether anesthesia (Moore, 1957; Sayers, 1957).

For blood glucose levels (Table 1) again, there was not a statistically significant trend in the regression lines for any treatment ($T < 2.3$). Although hyperglycemia is a usual consequence of stress, it reflects glucocorticoid secretion, and since cortisol levels did not increase, the present results with

TABLE 1. Plasma cortisol, blood glucose, and plasma chloride levels in the rainbow trout as a function of time anesthetized at 10 C. Each data point ($\bar{x}$) is the mean of three experiments. Correlation coefficient (r), t-test for zero slope (T), and standard error (SE) are given. The slopes of the calculated regression lines were not significantly different from zero ($T < 2.3$).

Exposure time (*min*)	Anesthetic											
	M.S. 222				Neutralized M.S. 222				Benzocaine			
	$\bar{x}$	SE	r	T	$\bar{x}$	SE	r	T	$\bar{x}$	SE	r	T
Plasma cortisol levels (μg %)												
3	18.6	8.5	−0.52	1.73	7.3	3.1	0.04	0.11	7.3	4.4	0.42	1.30
4	19.6	6.1			7.0	4.8			2.0	1.0		
5	14.6	6.8			5.0	1.2			5.3	3.4		
6	16.6	6.5			10.3	5.4			1.0	0.0		
7	14.8	4.4			4.5	3.1			4.0	0.7		
8	14.6	4.4			1.0	0.0			3.6	1.4		
9	15.6	3.1			8.0	6.3			6.5	3.1		
10	15.0	5.4			9.6	6.5			6.6	2.0		
11	9.0	10.0			7.0	3.1			10.6	5.8		
12	18.0	0.0			7.0	3.1			10.6	4.8		
Blood glucose levels (mg %)												
3	110.0	16.1	−0.31	0.92	78.3	3.0	0.36	1.10	107.7	13.3	−0.37	1.13
4	99.7	13.7			80.0	13.7			125.7	11.3		
5	110.0	26.3			91.0	15.7			119.7	16.1		
6	105.0	23.9			94.3	14.0			119.3	35.2		
7	128.3	48.2			94.5	5.6			124.3	27.3		
8	82.3	22.2			94.0	6.8			135.7	35.2		
9	105.0	36.9			83.3	8.9			113.0	35.5		
10	112.7	26.3			103.3	16.7			116.7	7.9		
11	96.0	12.5			78.0	8.2			117.0	40.6		
12	92.5	23.2			95.5	6.9			90.0	33.8		
Plasma chloride levels (mEq/liter)												
3	107.7	6.5	−0.29	0.85	111.0	4.8	−0.43	1.37	103.3	11.6	0.23	0.68
4	109.0	9.2			112.7	23.2			112.3	12.4		
5	114.0	5.5			108.3	12.0			118.0	11.6		
6	102.0	24.6			104.0	18.4			114.7	28.7		
7	102.3	14.0			111.0	3.1			114.0	10.9		
8	107.7	9.9			114.0	4.8			113.7	12.3		
9	102.3	18.1			114.3	14.7			104.3	17.4		
10	103.7	11.3			110.3	20.2			114.7	13.7		
11	110.0	15.0			103.0	14.7			118.3	7.5		
12	105.0	3.8			102.3	8.5			110.0	7.2		

blood glucose are to be expected. Under the experimental conditions employed, the catecholamine response was, of course, minimized. It should be noted, however, that high circulating glucocorticoid levels are not always associated with hyperglycemia in this species (Hill and Fromm, 1968).

Liver function, as measured by plasma cholesterol levels, was affected as shown in Fig. 2. Again, the slopes of the calculated regression lines were not significantly different from zero at the 95% confidence level. However, the M.S. 222 data were significant at about the 93% level and thus there

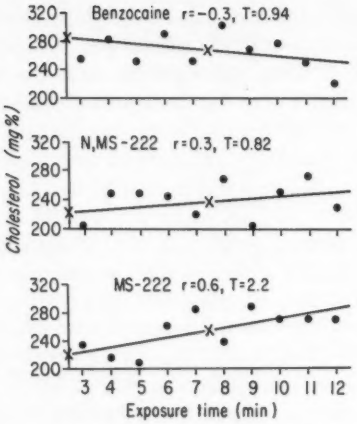

FIG. 2. Plasma cholesterol levels in rainbow trout as a function of time exposed to 0.03 mM benzocaine, neutralized M.S. 222, or M.S. 222, at 10 C. Correlation coefficient (r) and t-test for zero slope (T) are given.

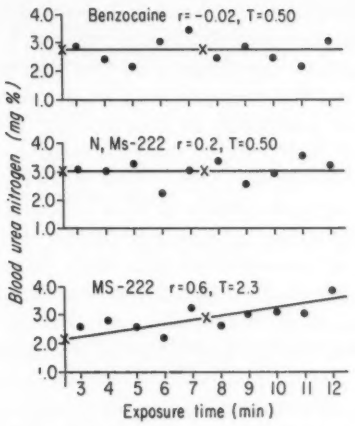

FIG. 3. Blood urea nitrogen levels in rainbow trout as a function of time exposed to 0.03 mM benzocaine, neutralized M.S. 222, or M.S. 222, at 10 C. Correlation coefficient (r) and t-test for zero slope (T) are given.

was some correlation ($r = 0.6$) between time anesthetized with M.S. 222 and plasma cholesterol level. This tendency toward hypercholesterolemia would probably bias cholesterol determinations somewhat if anesthesia was prolonged more than 5–6 min.

For blood urea nitrogen the data of Fig. 3 indicate that benzocaine and neutralized M.S. 222 had no effect: However, when the fish were anesthetized with M.S. 222, a progressive uremia developed that was significant at the 95% confidence level. Thus, BUN values determined on M.S. 222 anesthetized fish would tend to be biased upward if anesthesia was prolonged past a few minutes. Whether this trend is due to accelerated urea production or retarded excretion is unclear; however, it would seem logical that decreased gill transport due to the M.S. 222 (sulfonic acid) probably was not a factor, since plasma chloride levels were not affected (Table 1). In this case the data showed that no statistically significant trends in plasma chloride levels occurred with any of the three anesthetics used and the calculated regression lines had zero slope at the 95% confidence level.

An F test of variance ratios revealed that variability in the glucose, cortisol, and cholesterol data was greatest when M.S. 222 was used, whereas the variability in the chloride and BUN levels was independent of the anesthetic used. For interrenal ascorbate, neutralized M.S. 222 anesthesia gave the least variable results.

CONCLUSIONS

When rainbow trout are anesthetized with M.S. 222, metabolic changes occur that are correlated with exposure time. BUN levels progressively in-

crease, a tendency toward hypercholesterolemia occurs, and ACTH production as measured by interrenal vitamin C depletion, takes place. The calculated correlation coefficients for the regression of these changes on time are 0.6, 0.6, and 0.7, respectively, and are significant. These trends in blood chemistry though statistical in nature, reflect the stress of anesthesia due to the low *p*K of M.S. 222. Anesthesia with neutralized M.S. 222 (*p*H 7, free base) or benzocaine eliminated these disturbances in metabolism, implying that the sulfonic acid moiety of M.S. 222 is involved. The use of neutralized M.S. 222 or benzocaine also prevented the initial agitated swimming that occurs when fish are anesthetized with M.S. 222. In addition, benzocaine anesthesia was subjectively associated with less blood clotting and much less reflex twitching compared with the other anesthetics. Also, the variability in plasma glucose, cortisol, and cholesterol levels was greater with M.S. 222 than with neutralized M.S. 222 or benzocaine. Thus benzocaine or neutralized M.S. 222 would be the anesthetics of choice for clinical chemistry measurements, unless the exposure period was limited to 3–4 min or water of sufficient hardness ($CaCO_3$) to buffer the M.S. 222 to *p*H 7 could be used.

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Reading Scales to Age Pacific Cod (*Gadus macrocephalus*) from Hecate Strait

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Criteria for distinguishing annuli on the scales of Pacific cod, *Gadus macrocephalus*, are described. Evidence is presented that the criteria enable the ages of most Hecate Strait cod to be correctly determined. The method used to validate the scale-reading technique appears to be a new one; it may be applicable to other species.

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INTRODUCTION

THIS PAPER describes a scale-reading technique by which ages have been assigned to Pacific cod (*Gadus macrocephalus*) from Hecate Strait and assesses its validity. Although otoliths are used for determining the ages of Atlantic cod (*Gadus morhua*), many years of unsuccessful effort at this laboratory indicate that ages of Pacific cod taken by Canadian fishermen cannot be determined from otoliths, nor has any other method of finding their ages been developed until now. Noting that some investigators have determined the ages of Atlantic cod from scales, I tried a scale-reading technique with which I was familiar (having used it on several freshwater species) and found annuli-like features on Pacific cod scales.

The study is based on scales taken from Pacific cod caught by commercial trawlers in Hecate Strait from September 1967 to August 1968. Samples were taken on each of six dates, each sample (114–175 fish) being from the catch of one fishing vessel. Catches taken during the 2–7 days that the vessels fished had become mixed in the holds, so the mid-date of the period fished was recorded as the date of capture. About 50 scales were taken off each fish from about midway between the second dorsal fin and the lateral line. The scales were placed in a small coin envelope on which species, time, place, other particulars of capture, fork length (length from the tip of snout to fork of caudal fin when the fish was extended on a flat surface), and a serial number, were recorded. Sex was not recorded because the fish had been gutted at sea.

THE TECHNIQUE

In the laboratory, envelopes were discarded if practically all the scales in them were regenerate or quite atypical in shape, or if records were suspect. From the 105–165 envelopes remaining per sample, 100 were taken at random and scales from them were read. An age was assigned to every one of the 100 fish regardless of difficulties or doubts. The scales from some fish required up to 2 hr of intensive study, whereas others took only a minute or two; usually

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ages were assigned to about five fish per hour. Each age assignment was based on at least three scales, more unless it was readily apparent that the ages derived from all three were the same.

The scales were read by means of an Eberbach microprojector, a commercial version of the apparatus described by Van Oosten et al. (1934), at a magnification of 42. Scales were mounted just before examination. From each scale envelope about 15 scales were mounted between two glass slides with water, a minute proportion of detergent having been added to the water as a wetting agent. The scales were not cleaned since enough were mounted that the few obscured by dirt could be disregarded.

In many instances, but not all, it was easier to distinguish annuli when the mounted scale was "rough" side down on the stage of the projector. The slight roughness on one side of a scale resulted from surface irregularities that were identified visually as circuli or sclerites. The rough side could be identified macroscopically by feel, and by pigmentation; it was the outer side of the scale in life. When it was down the observer saw the pattern of circuli directly, but when it was up he saw it through the thickness of the scale.

After the annuli of several scales on a given slide had been identified and counted, an index card was laid along the dorsoventral diameter of the magnified image of one typical scale. The position of scale centre, of each annulus, and of scale edge were marked on the edge of the card. The distances between marks were later measured and recorded; the recorded values were of course proportional to distances between inner annulus and scale centre, between consecutive annuli, or between outer annulus and scale edge.

Characteristics of Annuli

In all instances the thing that I call an annulus was a boundary within which nearby circuli were notably closer together than those immediately outside it, and along which were the broken ends of circuli, in contrast to their usual pattern of concentric rings. The false annuli (summer checks) found on many of the scales had the same characteristics, although (unlike annuli) they usually could not be traced through all four quadrants of the scale. An annulus always coincided with the locus of a *slight* depression in the scale surface that could be found by carefully manipulating the focal plane. In case of doubt, an "annulus" was accepted or rejected on the basis of presence or absence of such a depression.

ASSESSMENT OF METHOD

Qualitative Seasonal Changes Near the Scale Edge

Figure 1 shows a scale from a cod caught in late June on which are three annuli. The distance between the outer annulus and the scale edge (i.e., the margin) represents growth during the year of capture, and the wide spacing of circuli near the edge indicates rapid growth just before capture. Figure 2 shows a scale from a cod also taken in late June on which are five annuli and a margin that represents growth during the year of capture. Figure 3 shows a scale from a fish caught in mid-April with two manifest annuli and an incipient annulus on the very edge of the scale. The scale is interpreted as showing that the fish had not grown since growth slowed down (as indicated by closely spaced circuli) and eventually stopped during the fall or winter prior to capture. Such an incipient annulus would not become distinguishable as an annulus until growth had resumed.

All August scales showed well-defined margins usually consisting entirely of widely spaced circuli. September margins were similar, although the outer circuli tended to be closer together. October and January scales were almost identical to one another, and the circuli near the edge in both were typically still closer together than on September scales. Most April scales were much

FIG. 1. Scale from a Pacific cod whose fork length was 54 cm when captured on June 22, 1968, in Hecate Strait. There are three annuli: they are respectively at 48, 72, and 94% of the distance from the scale centre to its edge.

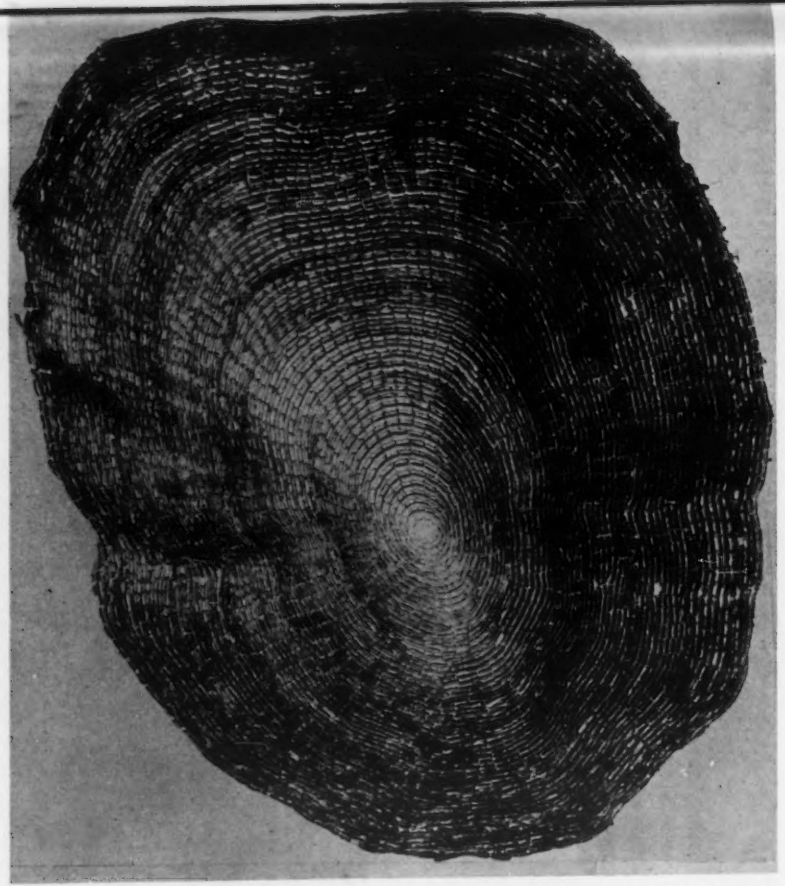

FIG. 2. Scale from a Pacific cod whose length was 71 cm when captured on June 22, 1968, in Hecate Strait. There are five annuli: they are respectively at 22, 43, 59, 76, and 89% of the distance from the scale centre to its edge.

FIG. 3. Scale from a Pacific cod whose fork length was 54 cm when captured on April 18, 1968, in Hecate Strait. There are two distinguishable annuli plus an incipient annulus on the very edge of the scale. The distinguishable annuli are respectively at 37 and 77% of the distance from the scale centre to its edge.

like October and January scales although typically subtly different, often giving the impression that there must be an incipient annulus on the very edge as in Fig. 3. A few April scales had margins although none were as wide as in Fig. 1 and 2. June scales typically showed about as much margin as seen in Fig. 1 and 2, but a few were like the scale in Fig. 3. These observations are interpreted as meaning that Pacific cod from Hecate Strait grow little, if any, during the winter and early spring; that individual cod differ among themselves considerably with respect to the date on which seasonal growth resumes, a few having already started by mid-April, and a few having not yet started by late June; that they grow mainly during the summer; and that they grow little, if any, after October.

Early summer scales required special caution because a comparatively wide margin could have resulted from either (1) above-average seasonal growth during year of capture, or (2) below-average seasonal growth during the year before capture with no growth during year of capture. The spacing of circuli near the edge of the scale is the best guide to proper interpretation when both possibilities exist.

Agreement Among Readings

To test the reproducibility of results, four samples were read twice by the same reader at intervals of more than 1 month. The frequencies with which the age assigned at the second reading differed from that assigned at the first were as follows:

Age difference	−3	−2	−1	0	+1	+2	Total
No.	1	2	34	332	28	3	400

Thus 85% of the ages assigned in the two sets of readings were identical and 98.5% of them differed by not more than 1 year.

I later trained a technician to read Pacific cod scales. She consistently has 91% agreement between reading and rereading, and 90% agreement between her readings and mine.

Seasonal Changes in Scale Margin Width

Measurements that had been taken during scale reading were used to find the ratio (ratio A):

$$\frac{\text{Distance from outer annulus to edge of scale}}{\text{Distance from outer annulus to next-to-outer annulus}}.$$

A value for ratio A was calculated for each of the 600 fish to which ages had been assigned. The ratio was a measure of the amount that each scale grew between the first of the year and the time of capture relative to its potential growth in a whole year as indicated by its growth during the calendar year before capture. Average values of ratio A for each of the six samples of 100 fish were: April 18, 0.052; June 22, 0.324; August 6, 0.523; September 10, 0.580; October 16, 0.714; January 13, 0.723.

tance from the scale centre to its edge.
Kennedy — J. Fish. Res. Bd. Canada

For calculating the above, the incipient annulus theoretically present on the very edge of each January scale was disregarded, and everything outside the outer manifest annulus was treated as scale margin. But if an April or June scale had an incipient annulus, the scale margin width was taken as zero. Data classified by age had been pooled only after analysis of variance had failed to demonstrate a significant difference[1] among averages of ratio A by age.

The average values of ratio A derived from the October and January samples, respectively, did not differ significantly. Therefore, the two samples were pooled and their combined average, 0.718, was treated as an estimate of the average value of ratio A at the end of the growing season, i.e., when scale margin had attained ultimate width. So on the basis of the text above, the scales made about 7% of their potential annual growth by April 18, 45% by June 22, 73% by August 6, 81% by September 10, and 100% by October 16, after which there was essentially no growth until the following spring.

There would have been no such pattern of steady marginal growth if an appreciable proportion of summer checks within scale margius had been incorrectly identified as annuli. Marginal growth for the misidentified group would have reached a maximum in midsummer as well as in October, so that if many had been misidentified, the sample average would have been affected.[2] The pattern of steady growth indicates that very few, if any, summer checks laid down during the year of capture were incorrectly identified as annuli. In other words, no more than one annulus could have been formed during the year preceding capture.

Ultimate Width of Scale Margin Compared with Distance Between Annuli

Measurements taken during scale reading were used to find the ratio (ratio B):

$$\frac{\text{Distance between annulus } x+1 \text{ and annulus } x+2}{\text{Distance between annulus } x \text{ and annulus } x+1}.$$

A value for ratio B was calculated for each of the possible sets of annulus-to-annulus measurements for all 600 fish, yielding one value (when $x = 0$) for each age-2 fish, two values (when $x = 0$, and when $x = 1$) for each age-3

[1] $P = 0.05$ in all instances where the significance of a difference is reported in this paper.

[2] When marginal growth values are plotted against time (Fig. 4), the point for August falls above, and the point for September falls below a single smooth curve (not drawn in Fig. 4) through all the points, suggesting that some summer checks laid down between August and September had been misidentified. However, a preferable alternative interpretation is that there are really two smooth curves as in Fig. 4. In any case I have, with crude mathematical models, assessed theoretically the effects of various proportions of such misidentified summer checks. It appears: (1) that if more than 12% were misidentified, the data would indicate negative growth between August and September; and (2) that the departure of the August and September points from a smooth curve would be consistent with 5% or less misidentification.

fish, etc. Ratio B was a measure of scale growth during the fish's x + 2nd year relative to growth during the previous year. Except for such April and June scales as were judged to have an incipient annulus on the edge, only manifest annuli were recognized. Also "annulus x" in ratio B was interpreted as "scale centre" when $x = 0$.

An average value of ratio B was found for each value of x. Analysis of variance showed that the averages did not differ significantly from one another except where $x = 1$. Ratio B averaged 0.717 when $x = 1$, and 0.816 when $x = 0$, 2, 3, or 4. The two averages differed significantly.

The average of ratio B when $x = 1$, i.e. 0.717, was not significantly different from 0.718, the ultimate value of relative scale margin width calculated in the previous section. In other words, relative scale margin width increased ultimately by about the amount that scale width increased during the fish's 3rd year of life (x + 2nd year with $x = 1$) based on annulus-to-annulus measurements. It seems unlikely that the two averages would be so nearly alike had appreciably more summer checks been misidentified between the second and third annuli than within the scale margin, because distances between annuli and false checks are necessarily less than distances between neighbouring annuli; therefore, appreciable misidentification would change ratio B. Thus not more than one annulus could have formed during the 3rd year in most instances, if not all.

Plotting relative scale margin widths against time (Fig. 4) reveals a need for two curves, one to represent the slower marginal growth of fish in the September, October, and January samples, the other to represent the faster marginal growth of fish in the April, June, and August samples. The upper curve was extrapolated (as a broken line) on the assumption that growth pattern would be the same, except for faster growth, as that represented by the lower line, i.e., that observed growth rate would be maintained until about the end of September, then soon slow to zero. Since 0.816 is the value of ratio B when $x = 0$, 2, 3, or 4, it is pertinent to ask: should the extrapolated line become horizontal at a level on the graph corresponding to 0.186? If the given assumptions govern extrapolation, then the level at which the extrapolated line becomes horizontal must indeed be reasonably close to 0.186. Thus the extrapolated line representing April, June, and August samples tends to become horizontal at about 0.816 just as the solid line representing September, October, and January samples becomes horizontal at 0.717, the average of ratio B when $x = 1$.[3]

[3]The September, October, and January samples consisted mainly (64, 82, and 73% respectively) of fish in their 3rd year. Since the ultimate value of ratio A (0.718) is determined mainly on the basis of these samples, it is not surprising that it is closer to the value for ratio B when $x = 1$ (0.717) than to the value when $x = 0$, 2, 3, or 4. The April, June, and August samples consisted mainly (95, 91, and 79% respectively) of fish that were not in their 3rd year. The horizontal part of the extrapolated line represents the ultimate value that ratio A would have had if it could have been based on the April, June, and August samples. It is not surprising that it should be closer to 0.816, the value for ratio B when $x = 0$, 2, 3, or 4 than to the value when $x = 1$.

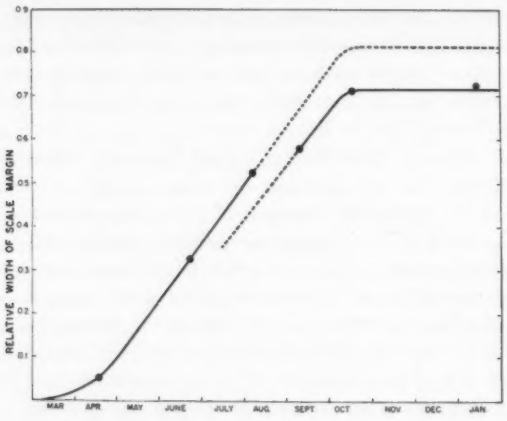

FIG. 4. Seasonal change in the relative width of margins on scales from Hecate Strait cod where their relative width on a given date is the average value of the ratio:

$$\frac{\text{Distance from outer annulus to edge of scale}}{\text{Distance from outer annulus to next-to-outer annulus}}.$$

This ratio really compares the average amount that scales grew between January 1 and date of capture with the average amount that they can grow in a year as indicated by how much they did grow during the previous year. The relative values it yields are therefore suitable for comparing growths made between January 1 and various dates of capture. The points represent average values of the ratio by date of capture; the solid lines are curves fitted to the points by eye, and the dotted lines are extrapolations.

Accepting that the level at which the extrapolated line becomes horizontal is about 0.816 is equivalent to acknowledging that essentially no more than one annulus is formed during years other than the third (x + 2nd year when x = 0, 2, 3, or 4). For, on the basis of the same argument used in the second last paragraph it seems unlikely that ultimate scale diameter would be approximately the same as the distance between successive annuli (3rd year data being excluded in each instance) if appreciably more summer checks had been misidentified between annuli than within the scale margin. So, to the extent that reliance could be placed on extrapolation, the data indicated formation of not more than one annulus per year. In any case, it was unlikely that summer checks would have been correctly identified both in the margin and between the second and third annuli, but not elsewhere on the scale.

Analogous reasoning also indicates that ultimate marginal width would likely not have been essentially the same as distance between annuli if less than one annulus had been formed per year.

In summary, then (in most, if not all instances): (1) not more than one annulus was formed during the 3rd year; (2) not more than one annulus was formed during years other than the 3rd; and (3) not less than one annulus was found during any years. Therefore most, if not all, of the fish used must have formed one, and only one annulus per year. In other words annuli must have been correctly identified in most if not all instances.

Length–Age Relation

Examination of the relation between fork lengths and the ages assigned by scale reading showed an orderly increase in size with age such as would be expected with correctly assigned ages. Average sizes by age were essentially the same as those estimated by Ketchen (1964) by another method.

As the ages in each sample differed from those in any other by some fraction of a year the lengths were adjusted to those expected if all the fish sampled had been caught on their nearest birthday. An appropriate amount was subtracted from each recorded size in pre-July samples, or added to each recorded size in post-July samples. The amount added or subtracted was calculated on the basis of the percentage of a year's growth attained by the sampling date, and of the average length increment at each age; fork length was assumed to increase by the same fraction as the scale diameter.

The length–age distributions based on combined data for all samples after such adjustments (Table 1) showed that length distribution was related to age, even though the full extent of the relation may have been obscured by truncated length distributions; there can be little doubt that smaller fish

Table 1. Frequency distributions of Hecate Strait cod lengths according to ages assigned by scale reading. Fork lengths, adjusted to the values expected had the fish been caught on their nearest birthday, are grouped by 25-mm intervals, the midpoints of which are shown.

	Age					
Fork length (*mm*)	2	3	4	5	6	7
385	1	0	0	0	0	0
410	0	0	0	0	0	0
435	0	0	0	0	0	0
460	0	4	0	0	0	0
485	3	17	1	0	0	0
510	3	33	3	0	0	0
535	3	54	4	0	0	0
560	3	66	8	0	0	0
585	3	54	10	0	0	0
610	1	73	20	0	0	0
635	2	33	22	0	0	0
660	0	21	19	4	0	0
685	0	13	23	2	2	0
710	0	8	11	9	2	0
735	0	0	9	5	0	0
760	0	1	9	5	4	0
785	0	2	5	5	1	1
810	0	0	3	2	1	0
835	0	0	3	5	2	1
860	0	0	1	0	0	0
Total	19	379	151	37	12	2
Avg length	542	582	662	746	758	810

were underrepresented in the age 2 length frequencies, and larger fish may have been underrepresented in some or all of the age 5, 6, and 7 length frequencies. The changes with age of both length-frequency distribution and average length were consistent with reliable scale readings.

Ketchen (1964) calculated mean lengths of 24-month-old cod from Hecate Strait by probit analysis of length frequencies in samples taken by small mesh trawls, and estimated mean lengths of older fish (at year end) from the same area by considering changes in length of tagged fish between time of release and time of recapture. Ketchen's mean lengths (millimeters) compared with those of Table 1 were:

Age	2	3	4	5	6	7
Ketchen	435	552	642	711	764	805
Table 1	542	582	662	746	758	810

The difference between age 2 values may have resulted from underrepresentation of small fish in the Table 1 data. At each of the other ages the difference between averages is not greater than would be expected in view of all the circumstances.

CONCLUSIONS

The data available have indicated that: (1) scale margins increased in width steadily through late spring and summer; and (2) scale margin width by year-end was about the same as distance between successive annuli, when year of life was comparable. A line of reasoning based on these facts has indicated that most Hecate Strait cod, if not all, formed each year one and only one of what I have identified as an annulus. Also supporting a conclusion that annuli were correctly identified in most instances were: (1) agreement in most instances between two readings of scales from the same fish whether successive readings by one reader or readings by two readers were compared; (2) a credible increase in fork length as age increased; (3) agreement between average length–age values from scale reading and published length–age values based on methods that required no reading of scales or otoliths. It therefore seems that the scale-reading method used identified most annuli correctly.

ACKNOWLEDGMENTS

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Regional Variation in Muscle Myogen Polymorphism in Walleye (*Stizostedion vitreum vitreum*) as Related to Morphology

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UTHE, J. F., AND R. A. RYDER. 1970. Regional variation in muscle myogen polymorphism in walleye (*Stizostedion vitreum vitreum*) as related to morphology. J. Fish. Res. Bd. Canada 27: 923–927.

In a survey of walleye (*Stizostedion vitreum vitreum*) from several lakes in central Canada, starch-gel electrophoresis of muscle myogens showed only the three phenotypes previously reported from Lake St. Clair. Measurements of body ratios and counts of interneural and interhaemal spines and of vertebrae gave no morphological basis for distinguishing between the three protein phenotypes. Although phenotype frequencies varied from lake to lake, within lakes the observed frequencies appeared to agree well with those calculated according to the Hardy–Weinberg rule assuming control by two nondominant alleles. The geographical variation was also consistent with the idea of reinvasion of the sample area from two separate refugia.

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INTRODUCTION

THIS PAPER DESCRIBES an attempt to differentiate morphologically between three biochemically distinct types of walleye (*Stizostedion vitreum vitreum*) (Uthe et al., 1966). Careful examination of individual members of the sea cucumber (*Thonella gemmata*) following a similar finding of two biochemical populations by Manwell and Baker (1963) showed that this species was composed of members of two morphologically discrete, probably noninterbreeding populations — "sibling species." Muscle myogen polymorphism in fish is extremely rare; greenstripe rockfish (*Sebastodes elongatus*) (Tsuyuki et al., 1969), sablefish (*Anoplopoma fimbria*) (Tsuyuki et al., 1965), and longnose sucker (*Catostomus catostomus*) (Tsuyuki et al., 1967) are the only other known examples. The observation that Great Slave Lake probably contained only a single variant of the three types of *S. vitreum vitreum* observed in Lake St. Clair (Uthe et al., 1966) coupled

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with the finding that artificially produced salmonid hybrids gave a quite analogous picture (Tsuyuki and Roberts,1965) suggested the possibility that the Great Lakes *S. vitreum vitreum* might represent two formerly allopatric populations as suggested by Radforth (1944). Evidence for this hypothesis was sought by morphological examinations of a number of each variant from a number of Canadian lakes. Hardy–Weinberg distributions were calculated for each phenotype found in the various lakes and compared with probable frequency values to throw some light on the genetic nature of the muscle myogen polymorphism. For comparison with a closely related species, similar electrophoretic and morphological data were obtained for sauger (*Stizostedion canadense*).

MATERIALS AND METHODS

Approximately 200 specimens of *S. vitreum vitreum* were collected from various Canadian lakes, and 36 specimens of *S. canadense* from Lake Superior. Specimens from lakes Huron, St. Clair, Superior, and Great Slave were obtained through the facilities of the Fisheries Research Board. Lake Winnipeg fish were obtained through the cooperation of the Manitoba Department of Mines and Natural Resources. Baptiste Lake (eastern Ontario) walleyes were collected by the Ontario Department of Lands and Forests.

Samples of the muscle were taken for electrophoresis as follows. The portion of the musculature below the anterior dorsal fin spines, down to the backbone, was removed and frozen over dry ice. The rest of the carcass was fixed in formalin and stored in 40% isopropanol. Electrophoresis of muscle myogens was performed as described by Tsuyuki et al. (1965), using essentially the starch-borate system of Smithies (1955). Radiography was used to determine interneural and interhaemal spine counts as well as vertebral numbers. Standard measurements were determined (Hubbs and Lagler, 1958) and the following four ratios calculated to emphasize any variability that might occur: orbital length to interorbital width; standard length to head length; interpelvic width to width of pelvic fin base; upper jaw length to gape width. All specimens examined were mature so that these ratios would be relatively stabilized and comparable between samples.

RESULTS

Electrophoretically the specimens examined (Table 1) showed only the three phenotypes previously reported. It is obvious that the frequency distribution in Lake Winnipeg is different from those found in the Great Lakes. The majority of the Lake Winnipeg specimens were of the type (AA, Fig. 1) found in Great Slave Lake. With the exception of Lake Superior, no significant differences were found between the observed frequencies and those calculated from the Hardy–Weinberg proportion (Table 1) suggesting genetic control of this polymorphism by two nondominant alleles.

No basis was found to distinguish morphologically between the various electrophoretic types from any of the lakes studied, except between *S. vitreum vitreum* and *S. canadense*. The morphological ratios varied considerably more in lakes Superior and St. Clair than in Lake Huron specimens but neither in the same characteristics nor in the same direction (Table 2). The three types in Lake Huron were close to each other in each characteristic but the orbital one. Very little variation was found in the counts of interneural and inter-

TABLE 1. Geographical distribution of the muscle myogen polymorphism found in walleye, and Hardy–Weinberg frequency data assuming nondominant allele control of the phenotype.

Lake	No. specimens	Phenotype AA O[a]	AA E[a]	BB O	BB E	AB O	AB E	q^a	X^2
Winnipeg	61	54	54.131	0	0.195	7	6.551	.057	.226
Superior	31	4	6.761	6	8.773	21	15.404	.532	4.036
Huron	32	5	4.499	13	12.499	14	15.002	.625	.143
St. Clair	51	10	10.328	15	15.371	26	25.199	.549	.044
Baptiste	38	8	7.592	12	11.579	18	18.753	.552	.067

[a]O = observed; E = expected.

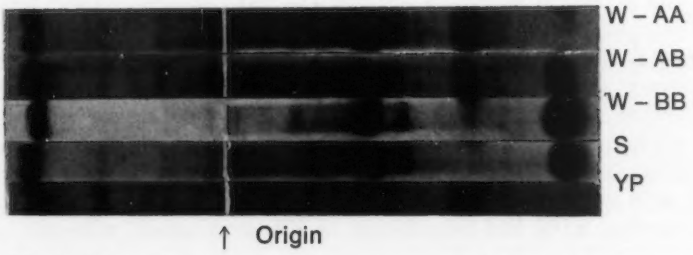

FIG. 1. Electrophoretic patterns of various members of the family Percidae. W-AA, W-BB, and W-AB, walleye; S, sauger; YP, yellow perch (*Perca flavescens*). The anode is to the right.

TABLE 2. Morphometric ratios (means and ranges) for the three phenotypes of walleye and a single type of sauger.

Lake	Morphometric ratio[a]	Phenotype			Sample size		
		AA	BB	AB	AA	BB	AB
Superior	1	1.14(1.12–1.20)	1.21(1.17–1.22)	1.18(1.00–1.32)	4	6	21
	2	3.12(2.96–3.21)	3.12(3.03–3.25)	3.18(2.98–3.32)	4	6	21
	3	1.31(1.13–1.45)	1.24(1.10–1.58)	1.21(0.92–1.47)	4	6	20
	4	1.56(1.45–1.70)	1.60(1.43–1.74)	1.58(1.26–1.79)	4	6	21
St. Clair	1	1.14(1.04–1.31)	1.22(1.12–1.42)	1.14(1.00–1.27)	3	8	12
	2	3.20(3.16–3.26)	3.26(3.19–3.35)	3.28(3.18–3.37)	3	8	12
	3	1.27(1.08–1.47)	1.16(1.00–1.30)	1.18(1.00–1.39)	3	8	12
	4	1.68(1.54–1.84)	1.75(1.60–1.92)	1.67(1.52–1.82)	3	8	12
Huron	1	1.10(1.00–1.23)	1.18(1.00–1.31)	1.25(1.06–1.50)	5	13	14
	2	3.30(3.28–3.32)	3.29(3.18–3.52)	3.29(3.19–3.43)	5	13	14
	3	1.12(0.90–1.26)	1.09(0.80–1.33)	1.10(0.84–1.30)	5	13	14
	4	1.41(1.29–1.62)	1.47(1.27–1.98)	1.45(1.24–1.83)	5	13	14
Winnipeg	1	1.20(1.00–1.75)		1.03	36		1
	2	3.26(3.04–3.54)		3.35	36		1
	3	1.08(0.90–1.43)		1.33	36		1
	4	1.55(1.35–1.67)		1.58	36		1
Superior[b]	1	1.11(1.00–1.52)			36		
	2	3.34(3.06–3.51)			36		
	3	1.54(1.36–1.75)			36		
	4	1.47(1.16–1.95)			36		

[a]The ratios are as follows: (1) orbit length/interorbital width; (2) standard length/head length; (3) interpelvic width/width of the base of the pelvic fin; (4) upper jaw length/width of gape.

[b]Data for sauger (*S. canadense*).

haemal spines and of vertebrae. Certainly no significant differences in counts occurred between electropherogram types. Only sauger (*S. canadense*) stood out as being significantly different from *S. vitreum vitreum* in these counts. All raw data in this study is available upon request.

DISCUSSION

No morphological evidence was found to indicate that the muscle myogen polymorphism found in *S. vitreum vitreum* results from interbreeding of two morphologically distinct populations. Muscle myogen polymorphism is, in any event, a rather rare occurrence in fish. Usually muscle myogens appear to show good species specificity, the main exception being the identicalness of patterns in related species (Uthe and Tsuyuki, 1968; Westrheim and Tsuyuki, 1969) rather than polymorphism within a species.

Although in many instances the sample size is small, Hardy–Weinberg treatment of the data shows that the observed frequencies are in fairly good agreement with those predicted assuming genetic control by two nondominant alleles. The same type of allele control has been suggested for *Anopoploma fimbria* by Tsuyuki and Roberts (1969).

One possible explanation of this polymorphism is an entrance of *S. canadense* characteristics into the *S. vitreum vitreum* genetic pool. Natural hybridization has been reported by Stroud (1948) but this has not been confirmed and the two species commonly occur together without hybridization (Trautman, 1957). It would be doubtful, however, if this mechanism would only transmit the myogen characteristic with no reflection in morphological characteristics when such characteristics are so different in the parents. Stroud's (1948) hybrids reflected morphological characteristics of both parents. Also in a series of artificial salmonid crossings none of the offspring resembled precisely either parent but were somewhat intermediate (D. P. Scott, personal communication). In this regard, although the *S. canadense* myogen pattern resembles type BB, this type is very rare in Lake Winnipeg, the lake with the largest *S. canadense* population of any of the lakes studied. Also, Baptiste Lake, although having type BB in abundance, has no *S. canadense* in its drainage system.

There is even a greater possibility that the polymorphism may be a result of introgression between *S. vitreum vitreum* and *S. vitreum glaucum* (the blue pike). Unfortunately no specimens of *S. vitreum glaucum* could be obtained for study. Regier et al. (1969) comment on reports of naturally occurring hybrids between these two subspecies. Clark (1955) showed crossing in the laboratory and survival at least to age I. But the occurrence of the AB type in Lake Winnipeg casts doubt on this explanation since *S. vitreum glaucum* is reported in only lakes Erie and Ontario (Slastenenko, 1958; Scott, 1967; Regier et al., 1969). Hubbs and Lagler (1958) state that the blue-colored walleyes found in other regions are probably not identical with *S. vitreum glaucum* but that the situation is unclear and needs further study. Regier et al. (1969)

state that blue-colored walleyes from lakes other than Erie and Ontario are blue due to the mucus that partially shields the more normal yellow color.

Genetic isolation in the past provides a more likely explanation for the muscle myogen polymorphism in *S. vitreum vitreum*. The possible existence of both Atlantic and Mississippi refugia for *S. vitreum vitreum* during the last major glacial advance (Radforth, 1944) would have resulted in allopatric populations with distinct gene pools. Reinvasion of *S. vitreum vitreum* into Pleistocene lakes from the two refugia would likely result in a merging of the two gene pools especially in the Great Lakes region, which derived its fauna from both refugia. The two basic muscle myogen types (AA and BB) found in *S. vitreum vitreum* as well as the combined AB type are common in the Great Lakes and stocks derived from the Great Lakes (Baptiste Lake). The BB type, however, was not found in either Great Slave Lake (although the sample was small the probability of obtaining six specimens of a single type where two types occurred in equal proportions is only about 0.016; where the three types occurred, about 0.090) or Lake Winnipeg (except in the AB form) both of which probably have obtained their *S. vitreum vitreum* from the Mississippi refugium. The implication then, is that the BB type originated in the Atlantic refugium and the AA type in the Mississippi refugium, the AB type being found where postglacial connections between the two refugia occurred, or more recently through the agency of genetic drift. It should be pointed out that completely analogous findings have been reported for the muscle myogens of *Catostomus catostomus* (Tsuyuki et al., 1967).

That the three muscle myogen types do not represent reproductively isolated stocks of *S. vitreum vitreum* today is demonstrated by data from the Baptiste Lake complex. There, five discrete (on the basis of tagging data) spawning populations were sampled during the spring spawning runs when they were geographically segregated. All three muscle myogen types were found in four of the five populations (Table 3). The fifth one was represented by only two specimens but even here two muscle myogen types occurred.

TABLE 3. Numbers of fish representing muscle myogen types found in five walleye stocks at spawning time in the Baptiste Lake complex.

Spawning location	Type		
	AA	BB	AB
McGarry Cr.	1	5	5
Hamilton Bay	2	2	3
Riffle	2	3	4
Elephant L.	3	1	5
West Bay	0	1	1
Total	8	12	18

In conclusion we do not think that the muscle myogen polymorphism is due to hybridization between *S. vitreum vitreum* and any other species but is simply a reflection of the well-documented protein heterogeneity found in many species of animals, which in turn may be related to its genetic history.

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Body Composition Changes During Growth in Young Sockeye (*Oncorhynchus nerka*) in Fresh Water

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The body composition of young sockeye (*Oncorhynchus nerka*) in the weight range of 0.5–300 g was investigated to further document changes in body composition with growth, and to develop equations that would allow the estimation of total body composition in this species, either in vitro or in vivo, based on easily measured parameters such as fork length, live weight, and total body water. The major components of the fat-free mass (protein, water, and ash) in nonstarving fish were closely related to each other and to fork length. Body fat tended to increase with size, but was not closely related to the other components of body composition. The following equations were derived, allowing the accurate estimation of total water (W), protein (P), and fat-free dry material (FFDM, consisting of protein and ash) in grams, based on the measurement of either water or fork length (L) in centimeters: $W = 0.00571\ L^{3.118}$, $P = 0.204\ W^{1.028}$, and $FFDM = 1.113\ P$. Body fat (F) may be estimated as the difference between wet body weight (M) and the lean mass: $F = M - (W + FFDM)$.

These equations are similar to, but not identical to, the relations derived for other species of salmon, based on other published data, and to the respective relations reported in the literature for mammals. The applications of these equations to the nutritional evaluation of wild populations and to quantitative nutritional studies on young sockeye are discussed and illustrated.

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INTRODUCTION

SINCE EARLY in the present century, many studies on mammalian body composition have been carried out, particularly on domestic animals (McMeekan, 1940; Murray, 1922; Wood and Groves, 1965; Widdowson et al., 1960), small laboratory species (Bailey et al., 1960; Widdowson and McCance, 1957; Bender and Miller, 1953b), and man (Pace and Rathbun, 1947; Keys and Brozek, 1953; Benke, 1963). The similarities between species in the relation of the various gross chemical components of body composition (fat, protein, water, and ash) and in the relative changes in these components with physiological maturity have served as the basis for concepts of chemical development in the mammals (Moulton, 1923). Bender and Miller (1953a, b) and Miller and Bender (1955) demonstrated the close relation between total body nitrogen and body water in the rat and used this relation as the basis for a rapid method of evaluating dietary proteins. Bailey et al. (1960) from studies on the body composition of the Swiss mouse during growth suggested that the protein:water ratio is a measure of physiological age in that species. All of these studies indicated that the composition of the lean body mass, particularly with respect to water and protein, changes in a

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regular and dynamic way with maturity but that these changes are not necessarily well correlated with the passage of chronological time. Body fat tends to increase with age, but changes in the amount of fat are much more closely related to the sufficiency or nutritional imbalance of the diet than to changes in the lean mass. Detailed chemical studies on mammals led to the development of allometric equations for the indirect computation of body composition (Bailey et al., 1960; Robertson, MS, 1957; Wood and Groves, 1965; Benke, 1963).

A number of studies on body composition in different salmonid species have been reported (Kizevetter, 1948; Parker and Vanstone, 1966; Vanstone and Markert, 1968; Idler and Clemens, 1959), but the changing relations between the various major chemical components of the body during growth are not as well understood in the fish, and generally applicable predictive expressions relating total protein, fat, water, and ash have not been derived.

Proximate analysis data for sockeye and other species of *Oncorhynchus* were examined to further document changes in body composition with growth, and to develop equations that would allow the estimation of total body composition in salmon based on easily measured parameters such as fork length, live weight, and total body water.

MATERIALS AND METHODS

Fish

Body composition data are presented for seven lots of young sockeye falling within a weight range of 0.5–350 g. The sources and previous treatments of these fish are summarized as follows:

Lot A consisted of four samples of Babine Lake sockeye fry sent in dry ice to the University of Victoria during the summer of 1968 by Dr D. Narver (Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C.). Each sample contained 100 individual fish. Three groups containing eight fish were removed from each of the four shipments for proximate analyses.

Lot B consisted of 28 groups of frozen yearling sockeye, previously used in trials at the Nanaimo Biological Station, in which the effects of temperature, feed intake, and exercise on growth were investigated during the summers of 1967 and 1968. Further details of these experiments are described by Brett et al. (1969).

Lot C consisted of 30 yearlings obtained from the culture facilities at the Nanaimo Biological Station in May 1968 and subsequently maintained at the University of Victoria. These fish were killed for analysis at intervals during the summer of 1968.

Lot D consisted of 11 two-year-olds maintained and sampled similarly to lot C in 1968.

Lot E was made up of 44 underyearlings, yearlings, and 2-year-old sockeye sampled directly from the culture tanks at the Nanaimo Station in February of 1969.

Lot F consisted of a total of 50 underyearlings, yearlings, and 2-year-olds maintained and sampled similarly to lots C and D during the summer of 1969.

Lot G consisted of 34 yearling sockeye taken at random for proximate analyses from a larger group of 180 fish used in nutritional studies at the University of Victoria during the summer of 1969. Some further details of part of these studies are provided in the footnotes to Table 7.

From the above, it is evident that the body composition data presented are for a number of different year-groups representing a rather wide spectrum of physical and nutritional environments. For comparative purposes, use has also been made of the following sources of other published body composition data for salmon: Idler and Clemens (1959) for data on 20 mature sockeye taken in salt water at Lumni Island prior to the spawning migration; Parker and Vanstone (1966), untransformed composition data on young pink salmon (*Oncorhynchus gorbuscha*);

and a 4-year summary of the proximate analyses of fall chinook fry (*Oncorhynchus tshawytscha*) from 18 Washington and Oregon hatcheries and laboratories for the years 1961–64, inclusive (J. R. Brett, personal communication).

Chemical Analyses

Water was determined in the earlier samples of fresh or frozen fish by desiccation to constant weight in a forced air oven at 80 C. The carcasses were slit longitudinally along the sides and the belly with a scalpel. More recently, drying was carried out in a vacuum oven at 70 C. This last procedure gave moisture values identical to the previous method, but resulted in less oxidation of fat. After drying, the carcasses were weighed, then crushed into tared flasks and extracted three times with approximately 10 volumes of *n*-hexane. Each extraction took place over about a 12-hr period, with occasional shaking of the flasks. After extraction, the remaining solvent was removed in the vacuum oven at room temperature. The fat-free dry material (FFDM) was weighed and was then finely ground through a #60 mesh screen for the determination of protein and ash. Protein determinations were by the Association of Official Agricultural Chemists' micro-Kjeldahl method using triplicated 10-mg samples of FFDM (Horwitz, 1960) or by the Dumas method using a Coleman Model 29-A Nitrogen Analyser and duplicate 100-mg samples of FFDM. Total protein was estimated as $N \times 6.25$. The two methods gave equivalent results. Ash was determined by combusting aliquots of FFDM to constant weight in a muffle furnace at 650 C, according to the Association of Official Agricultural Chemists' technique. In very small fish, ash had to be determined with a pooled sample. Either the FFDM was dried for 1 hr in an oven at 100 C immediately before the aliquots were weighed for protein and ash determination, or else a moisture determination was carried out on separate 0.5- to 1.00-g aliquots of FFDM to correct for any moisture reequilibration during storage.

Statistical Analysis

Equations are presented summarizing the relations among the various components of gross body composition, and between these components and fork length. Whereas these relations could be adequately described over narrow weight and length ranges by means of second order polynomial equations, a more precise fit to the data was obtained over large ranges of size and weight through the use of simple exponential equations having the form $Y = ax^b$.

The equations listed in the tabulated results were calculated by least squares linear regression of the logarithmically transformed data. The error terms shown, expressed as percentages, represent the antilogs of standard errors of estimate for the corresponding decadic logarithmic equations. Further statistical evaluation of the data was carried out by methods outlined in Croxton and Cowden (1955) and Snedecor (1959).

RESULTS

The relations between total body protein and body water in the seven groups of sockeye analyzed in this study and in the composition data taken from the literature are summarized in Table 1. Equation 8, the composite equation for sockeye in the 2.5- to 300-g weight range, is compared in Table 2 with similar equations obtained for three mammalian species. In every case, there is a very high significant correlation between the logarithms of total protein and water. When the exponents of the equations in Table 1 were compared by means of Duncan's multiple range test (Snedecor, 1959), differences between exponents of ± 0.005 (comparison of pairs) or of ± 0.01 (for comparison of seven means) were significant at $P < 0.05$; therefore, the exponents of equations 1–7 inclusive differ significantly from each other. Since the error for equation 8, in which data for all sockeye analysed individually are combined, is only slightly greater than the errors of the individual group equations, it appears, however, that the differences among

TABLE 1. Relation between total protein (P) and total water (W) in growing salmon.

Equation no.	Lot	Species	Wt range (g)	N	Equation	± (%)	r	Data source[a]
1	A	Sockeye	0.5–4.0	12	$P=0.188W^{1.124}$	+6.8 −6.5	0.995	1
2	B	"	6.0–20.0	28	$P=0.145W^{1.195}$	+8.3 −8.0	0.983	1
3	C	"	2.5–7.0	30	$P=0.225W^{0.980}$	+6.0 −5.5	0.976	1
4	D	"	21–95	11	$P=0.161W^{1.113}$	+1.9 −1.7	0.999	1
5	E	"	2.5–120	41	$P=0.168W^{1.090}$	+3.5 −3.0	0.999	1
6	F	"	5.0–300	50	$P=0.205W^{1.046}$	+3.5 −3.0	0.999	1
7	G	"	24–60	34	$P=0.215W^{1.015}$	+5.0 −4.5	0.985	1
8	C–G	"	2.5–300	163	$P=0.204W^{1.038}$	+7.5 −7.3	0.997	1
9	—	"	2.5–2500	183	$P=0.197W^{1.054}$	+8.5 −8.2	0.990	1, 2
10	—	Pink	0.5–11.0	90	$P=0.202W^{1.090}$	+8.0 −7.4	0.995	3
11	—	Chinook	0.3–5.0	75	$P=0.139W^{1.090}$	+6.8 −6.4	0.978	4

[a]Data sources: (1) present study; (2) Idler and Clemens (1959); (3) Parker and Vanstone (1966); and (4) proximate analysis summary, Washington and Oregon Fish Hatcheries, 1961–64 (J. R. Brett, personal communication).

TABLE 2. Relation between total protein (P) and water (W) in several species.

Equation no.	Species	Live-weight range (g)	N	Equation (g *vs.* g)	±(%)	r	Data source[a]
8	Sockeye salmon	2.5–300	163	$P=0.204W^{1.038}$	+7.5 −7.3	0.999	1
12	Mouse	1–26	111	$P=0.139W^{1.27}$	+8.6 −7.9	0.990	2
13	Wistar rat	13–30	18	$P=0.040W^{1.51}$	+4.1 −3.9	0.961	3
14	Wistar rat (males)	40–100	33	$P=0.175W^{1.08}$	+3.7 −3.6	0.994	3
15	Wistar rat (females)	40–300	44	$P=0.138W^{1.14}$	+8.4 −7.8	0.994	3
16	Yorkshire pig	1,500–6,200	16	$P=0.028W^{1.26}$	+10.0 −9.0	0.991	4
17	Yorkshire pig	6,200–31,000	23	$P=0.029W^{1.09}$	+12.0 −11.0	0.974	4

[a]Data sources: (1) present study; (2) Bailey et al. (1960); (3) Robertson (MS, 1957); and (4) Wood and Groves (1965).

equations 3–7 represent fine structure on an overall relation that is very uniform in the weight range of 2.5–300 g.

The combination of equation 8 with data from Idler and Clemens (1959) for 20 mature sockeye taken from salt water and weighing approximately 2500 g yields equation 9, which does have a significantly different exponent,

but which is similar enough to equation 8 to suggest that the protein-to-water relation in the sockeye salmon is very uniform over a wide weight range. Equations 10 and 11 for pink and chinook salmon indicate similar relations in these species. Growth in the salmon, in the sense of protein and water is not very different from growth in mammals (Table 2).

Equation 18 (Table 3) is a composite protein-to-water relation that includes the data for groups A and B, which include wild fry from Babine Lake, and all but the starvation treatment groups described by Brett et al.

TABLE 3. Comparison of the relations of protein (P) and fat (F) to water (W) for growing salmon.

Equation no.	Lot	Species	Wt range (g)	N	Equation	±(%)	r	Data source[a]
18	A–D	Sockeye	0.5–95	87	$P=0.193W^{1.07}$	$+9.0$ -8.8	0.999	1
19	A–D	Sockeye	0.5–95	87	$F=0.171W^{0.84}$	$+78$ -43	0.836	1
10	—	Pink	0.5–11.0	90	$P=0.202W^{1.09}$	$+8.0$ -7.4	0.999	2
20	—	Pink	0.5–11.0	90	$F=0.471W^{0.95}$	$+60$ -38	0.808	2

[a]Data source: (1) present study; and (2) Parker and Vanstone (1966).

(1969). The environmental histories and nutritional states of the sockeye described by this equation are therefore very different. The relatively low error and high correlation coefficient for equation 18 contrast with the high error and lower correlation coefficient found for the fat-to-water relation for the same fish. The same contrast is seen between equations 10 and 20, calculated from the data of Parker and Vanstone (1966), for the pink salmon. Body fat tends to increase with body size, but this increase is not closely associated with increase in the lean mass. On the other hand, ash determinations carried out on 56 individuals in the 0.5- to 300-g weight range resulted in a value for ash of $10.19 \pm 1.69\%$ of the fat-free dry weight. The observed uniformity in the composition of the FFDM results in the following useful expression by which the FFDM may be estimated:

$$\text{FFDM} = 1.113\ \text{P}, \tag{21}$$

where P = grams of total body protein.

The relations of total protein and water to fork length for several groups of young sockeye in this study and for pink salmon (Parker and Vanstone, 1966) are summarized in Table 4. In this table, the least significant difference between pairs of exponents is ± 0.01, so that all of the exponents shown differ significantly.

Since length data were not recorded for all the groups used for proximate analyses, the relations of protein and water to length are not characterized by so many separate equations as are the relations of protein to water. As

TABLE 4. Comparison of the relations of total protein (P) and total water (W) to fork length (L) in centimeters in growing salmon.

Equation no.	Lot	Species	Wt range (g)	N	Equation	±(%)	r	Data source[a]
22	E	Sockeye	2.5–120	44	$P=0.00060L^{3.392}$	+4.8 −4.5	0.999	1
23	G	"	24–60	34	$P=0.000445L^{3.524}$	+8.0 −7.5	0.959	1
24	E, G	"	2.5–120	78	$P=0.000579L^{3.417}$	+7.6 −7.3	0.997	1
25	—	"	2.5–2500	98	$P=0.000874L^{3.256}$	+11.2 −10.2	0.998	1,2
26	—	Pink	0.5–11.0	90	$P=0.000370L^{3.630}$	+9.5 −8.4	0.999	3
27	E	Sockeye	2.5–120	47	$W=0.00580L^{3.105}$	+5.0 −4.8	0.999	1
28	F	"	5–300	31	$W=0.00655L^{3.082}$	+4.0 −3.6	0.999	1
29	G	"	24–60	34	$W=0.00174L^{3.571}$	+5.5 −5.2	0.981	1
30	E–G	"	2.5–300	112	$W=0.00571L^{3.118}$	+5.5 −5.2	0.998	1
31	—	"	2.5–2500	132	$W=0.00735L^{3.025}$	+7.8 −7.2	0.999	1,2
32	—	Pink	0.5–11	90	$W=0.0032L^{3.31}$	+8.0 −7.2	0.999	3

[a]Data source: (1) present study; (2) Idler and Clemens (1959); and (3) Parker and Vanstone (1966).

was the case with the equations in Table 1, significant differences between different group equations do not result in any great deviation of any of the data from the values estimated by the composite relations of protein and water to length (equations 24 and 30) applied over the body weight range of 2.5–300 g.

After the estimation of the components of fat-free mass (water, protein, and FFDM) on the basis of the relation of protein to water and the relation of protein and water to length, an estimate of total body fat (F) may be obtained by difference through the following expression:

$$F \doteq M - (W + FFDM), \tag{33}$$

where M = wet weight (g) and W = weight (g) of total body water.

In Table 5, fork lengths, wet weights, and body composition data for 12 individual fish randomly selected from groups C–G inclusive, are shown. The columns headed W_D, P_D, and F_D contain values for water, protein, and fat, respectively, obtained by direct chemical analysis. The columns headed P_W and F_W contain values for protein and fat, respectively, estimated from values of wet weight (M) and water (W_D) through the use of equations 21, 33, and 8. The columns headed W_L, P_L, and F_L, respectively contain values for water, protein, and fat estimated from values of M and length (L) through the use of equations 21, 33, 24, and 30. An alternative estimate of composition

TABLE 5. Fork lengths, wet weights, and measured and estimated body composition data for 12 individual young sockeye randomly selected from groups C–G.

Fish no.	Fork length L(*cm*)	Wet wt M(g)	Water		Protein			Fat		
			W_D[a](g)	W_L[b](g)	P_D(g)	P_W[c](g)	P_L(g)	F_D(g)	F_W(g)	F_L(g)
1	9.6	8.87	6.64	6.72	1.33	1.46	1.32	0.76	0.61	0.68
2	21.5	105.10	74.70	83.0	18.53	18.00	20.70	9.60	10.40	−0.95
3	21.0	103.5	73.90	77.10	18.17	17.80	19.10	9.33	9.78	5.13
4	15.5	42.80	30.10	29.90	6.63	7.00	6.75	4.94	4.90	5.38
5	12.6	20.70	15.00	15.70	3.21	3.39	3.33	2.22	1.93	1.29
6	28.0	263.0	181.8	189.0	46.8	45.4	51.0	25.4	30.7	17.2
7	28.5	289.0	201.9	200.0	53.1	50.5	54.1	24.9	30.8	28.8
8	11.7	15.70	12.12	12.40	2.79	2.71	2.59	0.29	0.56	0.42
9	6.9	2.86	2.26	2.40	0.41	0.48	0.43	0.15	0.07	−0.01
10	7.7	4.20	3.22	3.38	0.61	0.69	0.62	0.31	0.22	0.13
11	13.5	25.40	18.40	19.50	4.41	4.20	4.21	2.06	2.32	1.22
12	16.5	51.50	38.50	36.40	8.70	9.04	8.36	3.35	2.93	5.79

[a]Symbols having subscript D represent values obtained by direct determination.
[b]Symbols having subscript L represent values estimated from M and L through the use of equations 21, 24, 30, and 33.
[c]Symbols having subscript W represent values estimated from M and W_D, through the use of equations 8, 21, and 33.

could have been obtained through the use of equations 30, 8, 21, and 33. The chi-square values for the comparisons between measured and estimated values, $(W_L - W_D)$, $(P_W - P_D)$, $(P_L - P_D)$, $(F_W - F_D)$, and $(F_L - F_D)$, are shown in Table 6.

TABLE 6. Chi-square comparisons of estimated and measured variables for Table 5 (df = 11).

Comparison	Chi-square
W_L–W_D	1.589
P_W–P_D	0.281
P_L–P_D	0.741
F_W–F_D	3.066
F_L–F_D	15.964
Least significant value, ($P<0.05$)	19.675

DISCUSSION

It is difficult to say, on the basis of the present data, whether the differences between the group equations in Table 1 represent real physiological differences, or whether these differences were at least partially due to analytical artefacts. The error, for instance in protein determinations by the micro-Kjeldahl technique, on ground fat-free material was about ±1.0% in terms of the present protein in FFDM, or an error of about ±1.2% in

relation to total protein. The relatively low error in equations 8 and 9 suggests that sockeye, in the weight range of 2.5–300 g, or even in the extended range of 2.5–2500 g, do not undergo any very marked changes in the basic relation between protein and water. By comparison, the combination of equations 13 and 14 for small and large growing rats (Robertson, MS, 1957) or of equations 16 and 17 for small and large pigs (Wood and Groves, 1965) results in considerably increased error; the breaks in the protein vs. water curves for these species may represent genuinely different physiological relations.

Lipid determinations were carried out with somewhat lower precision than the protein determinations. The analytical error in this case amounted to about ±3% of the total amount of fat present. This level of error, however, cannot account for all of the increased variability of equation 19 as compared to the corresponding protein-to-water curve. Except for the small amount (in relation to total body fat) of lipid material normally found in totally lean tissue, there is no necessary physiological relation between fat and the components of lean mass. Fat tends to increase with body size, but is much more closely a function of the previous nutritional history of the fish. A similar result was observed by Vanstone and Markert (1968) and Parker and Vanstone (1966).

In a number of cases, the efficiency of the hexane extraction of total lipid was tested by reextraction of samples of finely ground FFDM with 10 volumes of water-saturated chloroform–methanol (3:1) (Folch et al., 1957; Bligh and Dyer, 1959; Ambrose et al., 1969). Such treatment removed an additional 3–5% of the residue remaining after the first series of hexane extractions, but this weight loss could be almost completely accounted for in terms of a loss of protein (N × 6.25). Had the nitrogen been assumed to derive from phospholipid, the corresponding weight loss (approx N × 56.5) would have been much larger than the weight loss actually observed. These findings were similar to those of Munro and Downie (1964). The nitrogenous material in the chloroform–methanol phase was removed by washing the latter with 0.005 M aqueous $CaCl_2$, followed by subsequent determinations of nitrogen in the resulting aqueous phase by the micro-Kjeldahl technique. Treatment of the above aqueous phase with an equal volume of cold 10% trichloroacetic acid did not produce a precipitate, suggesting that the nitrogen extracted was actually primarily peptide or amino acid nitrogen, possibly resulting from some autoproteolytic action in the initial stages of drying. A check of the efficiency of the hexane extraction showed that protein and ash determinations accounted for more than 99% of the FFDM. Although hexane is not a particularly good solvent for phospholipids, the hexane–triglyceride mixture obtained in the first hexane extract does remove the other lipids from the dry material. The hexane method was adopted because it seemed most satisfactory for a sequential determination of water, fat, protein, and ash in individual small fish.

The equations shown in Table 4 illustrate the approximately cubic relation between length and mass in fish described by Hecht (1916) and subsequently

recorded by a number of workers for salmonid species (Hoar, 1939; Parker and Vanstone, 1966; Vanstone and Markert, 1968). Equations 23 and 29 may be evidence of an upward break in the relations of protein to length and water to length at a length of about 12 cm, similar to the effects observed in coho by Vanstone and Markert (1968). The relations between the individual components of lean mass and fork length are quite uniform over wide weight and length ranges (equations 25 and 31).

An estimate of total body composition may be obtained using either the primary measured parameters of wet weight and fork length, and equations 21, 24, 30, and 33, or the primary parameters of wet weight and total body water (Table 5). The estimate of protein based on length is as precise as the estimate based on body water. There is a very close relation between the measured and estimated values for body water. The chi-square value (Table 6) for the comparison of measured fat (F_D) and estimated fat (F_L) based on length and weight is not significant, but is five times greater than the value obtained from total body weight and measured body water. This is to be expected, since F_L is based on more estimated parameters than F_W. Although the estimation of fat based on length may be somewhat inaccurate for single fish, this method allows a valid estimate of total fat in a group of fish, where the estimates for individual animals are summed. Body composition comparisons of "standardized" fish (Parker and Vanstone, 1966) are valid only if composition is first adjusted to the standard lengths by means of the individual component equations (i.e., 24 and 30), since the different components of lean mass change at different rates in relation to length. Errors in the measurement of fork length will be magnified, particularly in the case of estimated fat, because of the cubic relation between mass and length. In this respect, the negative values of F_L in Table 5 appear to derive from a positive error in measuring fork length.

Although equations 8, 21, 24, and 30 may have to be modified slightly as further analytical data are obtained, the present findings and those recorded in the literature for other species of salmon indicate that valid estimates of the body composition of salmonids may indeed be based on such readily measurable parameters as weight, length, and body water. Estimates based on weight and body water allow the most precise estimates for dead fish, whereas estimates based on weight and length measurements have application to in vivo studies. Both approaches greatly facilitate studies in which various nutritional regimens for growing salmon are assessed in terms of body composition changes.

The results of such a study, in which two commercial rations are compared with respect to their ability to support growth in yearling sockeye, are tabulated in Table 7. The estimates of body composition were made on the basis of fork length and body weight measured in vivo with anaesthetized fish. The details of the trial are indicated in the footnotes to Table 7.

The relative lack of change in shape with size in fish has been contrasted with the more complex changes in shape due to growth in mammals (Plásson,

TABLE 7. Estimates of protein, fat, and energy storage of yearling sockeye fed two different commercial rations [a,b] based on the application of equations 8, 21, 30, and 33 to measurements of fork length and body weight obtained in vivo.

Ration:	1		2	
Group:	1	2	1	2
Fork length (*cm*) — Initial	12.05	11.53	11.92	12.06
Final	15.10	14.60	16.32	16.52
Body wt (g) — Initial	18.4	15.8	17.8	18.6
Final	40.9	36.1	52.9	55.1
Protein (g) — Initial	3.01	2.59	2.77	3.02
Final	6.26	5.62	8.05	8.39
Fat (g) — Initial	1.62	1.37	2.36	1.82
Final	6.83	5.43	9.43	9.87
Gain — Wt (g)	22.5	20.3	35.2	36.5
Protein (g)	3.25	3.03	5.28	5.37
Fat (g)	5.21	4.06	7.1	8.1
Energy (*kcal*)[c]	67.6	55.7	96.8	106.4
Feed intake (g)	35.6	30.6	30.6	31.1
Protein intake (g)	15.6	13.4	19.9	20.2
Fat intake (g)	2.41	2.07	4.40	4.44
Wt gained/g feed	0.63	0.66	1.15	1.18
Protein gain/g feed protein	0.21	0.23	0.27	0.27
Fat gain/g feed fat	2.16	2.07	1.62	1.81
Energy in gain (*kcal*)/energy consumed (*kcal*)	0.42	0.42	0.62	0.67

[a]J. R. Clark Co., Salt Lake City, Utah. Before use, both rations were repelleted through a $\frac{1}{16}$-inch die using a California Type CL-3 laboratory model pellet mill. The proximate composition (by analysis) of the rations as fed is shown below.

	Ration 1	Ration 2
Moisture (%)	7.7	7.9
Protein (%)	43.4	64.9
Fat (%)	6.8	14.3
Carbohydrate (%)	30.4	3.1
Ash (%)	11.7	9.7
Gross energy (*kcal*)	4.4	5.1

[b]Uniformly sized yearling sockeye, 20 per group. The fish were anaesthetized with M.S. 222, and weighed and measured at the beginning of the experiment and again at the end of the trial 54 days later. Each group was maintained in a 140-liter enamelled iron bathtub equipped with an intermittent siphon overflow. Fresh water was sprayed through a fine inlet nozzle at a rate of 1.1 liter/min to provide aeration and stirring. The fish were fed ad libitum three times daily, and the enamel surfaces of the tanks were cleaned with a sponge once a week. The mean water temperature was 14 C.

[c]Gross energy values of fish and rations were estimated on the basis of 5.67, 9.4, and 4.2 kcal/g for protein, fat, and carbohydrate, respectively.

1955). In comparing his own results with data from even earlier studies such as those of Spencer (1871), Hecht (1916) stated that the "form of an individual fish is constant, within rather narrow limits throughout its life." For any particular species, variations in the equations relating weight to length,

having the form $W = a L^b$, occurred primarily in the factor "a" and resulted from differences in the "condition" of groups of fish sampled at different times. This is also illustrated by Vanstone and Markert (1968) whose data for juvenile coho show much greater variation in the coefficient a than in the exponent "b."

The present data and those of Parker and Vanstone (1966) suggest that in growing, sexually immature salmon, the primary variable in determining condition is body fat. Hecht's statement then would be still more descriptive if modified to read "the form of the fat-free mass of an individual fish is constant..." A necessary result of this concept and the present analytical data is that at any given fork length a nonstarving salmon of a given species contains a precisely specified amount of protein, water, and ash, and therefore has a closely predictable fat-free mass.

The fat-free mass (FFDM + water) of the sockeye is a function of fork length (Fig. 1, solid curve). Differences between predicted fat-free mass and the actual body weight represent differences in the amount of body fat, which

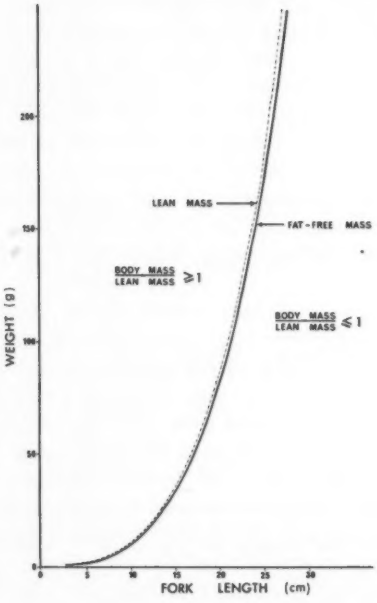

FIG. 1. Relation of lean mass and fat-free mass to fork length in sockeye weighing between 0.5 and 300 g.

in turn reflect the adequacy or inadequacy of the nutritional environment. In this respect, Brett et al. (1969) have demonstrated a close relation between percent body fat and the level of feed intake in young sockeye. A distinction should be made between fat-free mass and lean mass, which will contain a certain minimal amount of lipid material compatible with life. Analyses of starved young salmon (Parker and Vanstone, 1966; Brett et al., 1969) in-

dicated that even in severe starvation, the dry mass contains about 15% lipid. The predicted lean mass in grams of the salmon may therefore be estimated as follows:

$$\text{lean mass} = [\text{water} + (\text{FFDM} \times 1.15)]. \quad (34)$$

The predicted lean mass of growing sockeye as a function of fork length (Fig. 1, broken curve) could have application in the nutritional evaluation of wild populations, since fish receiving a maintenance level or better of energy intake will have a body weight:predicted lean weight ratio greater than or equal to one. A complete nutritional evaluation of such a wild population, however, also requires some attention to the length of the fish in relation to age, since an adequate intake of a ration high in protein and low in fat could result in rapid lean growth with only a small accumulation of fat, whereas an isocaloric intake of a low-protein ration would support less lean growth and a greater accumulation of fat.

The morphological and compositional changes involving physical elongation and a decrease of lipid considered to be typical of the parr–smolt transformation (Lovern, 1934; Hoar, 1939; Kizevetter, 1948; Parker and Vanstone, 1966) may result from growth in length and lean mass at the expense of stored fat in a nutritional environment providing some protein but being limited in energy. The absence of such a decrease in lipids during smoltification as observed in wild coho by Vanstone and Markert (1968) could occur if the diet of the fish during this period was adequate with respect to both protein and energy. In this respect, growth accompanied by a net loss of fat has been observed in this laboratory in 30- to 50-g sockeye fed submaximal quantities of high-protein diets.

Starving fish may have live weight:predicted lean weight ratios equal to or less than 1 (Fig. 1). In the lean, starving fish, body protein must serve as an endogenous energy source. If body fat is present, some protein must still be degraded to allow catabolic utilization of body fat. Whereas protein increases more rapidly than water in the lean mass of growing fish, in the starving fish both water and protein are lost, and protein disappears more rapidly than water. Thus not only are total water and protein less in a starving fish of given fork length, but the protein:water ratio of a starving salmon also tends to be less than for a growing one of the same length. These changes are illustrated by the composition data recorded by Idler and Clemens (1959) for mature Fraser River sockeye during the spawning migration and also by the data for starved juvenile pink salmon (Parker and Vanstone, 1966) and starved juvenile sockeye (Brett et al., 1969). The equations developed in the present study can therefore not be used for precise estimates of body composition in starving fish.

Although all of the data for young sockeye reported herein are for fish maintained in fresh water, the fact that the addition of data for composition of mature prespawning sockeye taken from sea water (Idler and Clemens,

1959) does not greatly alter the protein–water or protein–length and water–length equations suggests that these equations are probably valid for evaluating the composition of sockeye in salt water. Such an assumption, however, should be verified by further analyses of sockeye of various sizes maintained in salt water. A more extensive study of larger fish may also reveal some sex-dependent composition differences, particularly in more mature fish. Further differences in the equations presented would be expected between strains of sockeye having genuine genetic (as compared to environmentally imposed) differences in mature weight. It would be interesting to make parallel studies on normal sockeye and kokanee stocks sharing the same spawning and juvenile ranges.

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Electrophoretic Variants of L-Alpha-Glycerophosphate Dehydrogenase in Pacific Ocean Perch (*Sebastodes alutus*)

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JOHNSON, A. G., F. M. UTTER, AND H. O. HODGINS. 1970. Electrophoretic variants of L-alpha-glycerophosphate dehydrogenase in Pacific ocean perch (*Sebastodes alutus*). J. Fish. Res. Bd. Canada 27: 943–945.

Three phenotypes of L-alpha-glycerophosphate dehydrogenase (α-GPDH) were found in muscle extracts of Pacific ocean perch by starch-gel electrophoresis. No association was obvious between alpha-glycerophosphate phenotype and sex or stage of maturity. The observed phenotypes were presumed to be under the control of two allelic genes, F and S. This assumption was supported by the distribution of phenotypes of fish collected off the coasts of Washington and Oregon. This report is the first on genetic variants of an enzyme in Pacific ocean perch and suggests that isozymes of α-GPDH may be useful in population studies of this species.

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INTRODUCTION

THE PACIFIC OCEAN PERCH is one of 55 species of the genus *Sebastodes* along the northeast Pacific Ocean from Mexico to the Bering Sea (Phillips, 1957; Hitz, 1965; Westrheim and Tsuyuki, 1967; Strachan et al., 1968; Tsuyuki et al., 1968). Fish of this genus, particularly the Pacific ocean perch, are of increasing economic importance to the United States as well as to foreign fisheries; 8.8 million lb of *S. alutus* were taken by U.S. fishermen in 1960, whereas 28.4 million lb were caught in 1965 (Lyles, 1967). The genus *Sebastodes* is also of considerable taxonomic interest because of the large number of species; one species, *S. reedi*, has only recently been described (Westrheim and Tsuyuki, 1967) and others possibly remain undetected.

Tsuyuki et al. (1968), in a comparative electrophoretic study of hemoglobin, skeletal muscle, and lens proteins of 24 species of *Sebastodes*, detected apparent genetic polymorphisms in hemoglobin of *S. crameri* and in muscle of *S. elongatus*. These represent, to our knowledge, the only genetic variants reported in this genus.

L-Alpha-glycerophosphate dehydrogenase (α-GPDH), which catalyzes the reversible reaction dihydroxyacetone phosphate + NADH $\rightleftharpoons$ α-glycerophosphate + NAD^+, has been investigated by electrophoresis in several animals (Nyman, 1967; Scopes, 1968). Genetic polymorphism of this enzyme has been reported in *Drosophila melanogaster* (Grell, 1967).

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This paper is part of our laboratory's studies on certain unresolved population and taxonomic problems of the genus *Sebastodes*. We present evidence for a genetic system coding for α-GPDH in Pacific ocean perch that may be useful for population studies.

METHODS AND MATERIALS

Three lots of Pacific ocean perch, totaling 121 fish, were collected along the coasts of Washington and Oregon (Fig. 1). Whole fish were kept frozen until tested. Muscle samples were prepared by mashing them with wooden sticks in equal volumes of phosphate-buffered physiological saline (*p*H 7.4) into uniform pastes. Samples for electrophoresis were obtained without further treatment by immersing $\frac{1}{4} \times \frac{3}{16}$-inch filter paper (Schleicher and Schuell, grade S&S No. 470) wicks in the pastes and inserting them into starch gels.

Electrophoresis in starch gel followed the methods of Kristjansson (1963). The buffer system used was described by Markert and Faulhaber (1965). Gels consisted of 35 g starch plus 250 ml of gel buffer. A voltage of 300 was applied for 10 min; sample inserts were removed and 400 v applied until indicator dye markers reached a point 9.0 cm anodal to the origin (about 2.5 hr at 30–40 ma). Gels were cooled during electrophoresis either by placing ice packs on glass plates on top of gels or by electrophoresing the gels in a refrigerator.

After electrophoresis the zones of α-GPDH activity on the anodal part of the gel were made visible through the staining technique described by Nyman (1967). NAD^+ and substrate dependency were demonstrated by elimination of NAD^+ or substrate from the staining solutions.

PHENOTYPES OF α-GPDH IN PACIFIC OCEAN PERCH

Three phenotypes of α-GPDH were detected in muscle extracts of Pacific ocean perch (Fig. 2). These patterns may be explained by a two-allele hypothesis; the two single-banded phenotypes presumably reflect individuals homozygous for allelic genes, whereas the three-banded phenotypes reflect heterozygous individuals. The phenotype of the heterozygotes suggests that the active enzyme is a dimer, the middle band representing a hybrid molecule containing a subunit coded by each allele (Shaw, 1964). These patterns agree with other published reports of the molecular structure of α-GPDH (Grell, 1967; Klotz and Darnall, 1969).

After gene frequencies of the α-GPDH alleles were determined for each of the three collections of Pacific ocean perch, expected frequencies of phenotypes were computed, assuming a Hardy–Weinberg distribution (Stern, 1943) in the sampled populations (Table 1). Contingency tests indicated that the α-GPDH phenotypes were independent of sex and stage of maturity (Table 2). Phenotypes in two of the three collections and in the combined total did not deviate significantly from expected frequencies. The deviation from expected frequencies in the third (January 15, 1969) collection may merely reflect sampling error. Other reasons such as population heterogeneity or heterozygous advantage may be responsible.

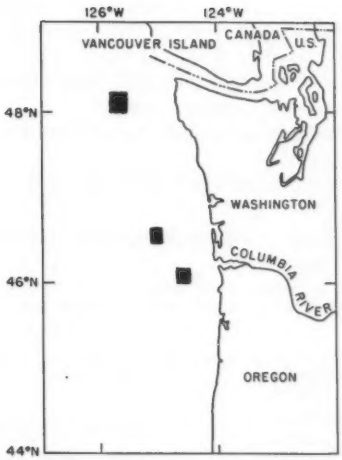

FIG. 1. Locations of *Sebastodes alutus* collections (solid squares), 1968–69.

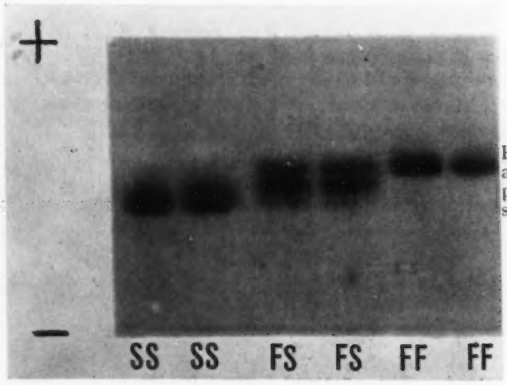

FIG. 2. Bands in starch gel illustrating alpha glycerophosphate dehydrogenase phenotypes (SS, FS, FF) in muscle samples from Pacific ocean perch, *S. alutus*.

TABLE 1. Frequencies of α-GPDH phenotypes in Pacific ocean perch collected off Washington and Oregon, 1968–69.

Collection date	Location		α-GPDH phenotypes[a]			Allele frequency		df	χ^2	*P* of chi-square
	N lat	W long	FF	FS	SS	F	S			
Nov. 22, 1968	48°00′	125°77′	11 (11.5)	12 (10.7)	2 (2.5)	0.680	0.320	1	0.14	>0.7
Jan. 15, 1969	46 40.6	124 47.5	4 (6.1)	13 (8.6)	1 (3.2)	0.583	0.417	1	4.49	>0.02
Jan. 22, 1969	46 02.8	124 42.1	33 (34.3)	37 (35.1)	8 (8.6)	0.660	0.34	1	0.19	>0.5
Total			48 (50.8)	62 (55.7)	11 (14.5)	0.65	0.35	1	2.06	>0.1
Chi-square of sums			–	–	–	–	–	3	4.82	>0.1
Hetero-geneity			–	–	–	–	–	2	1.75	>0.3

[a]Observed and in parentheses expected frequencies.

TABLE 2. Contingency test for relation of α-GPDH phenotype (FF, FS, and SS) to sex and stage of maturity for Pacific ocean perch collected off Washington and Oregon, 1968–69.

	FF		FS		SS					
	Observed	Expected	Observed	Expected	Observed	Expected	Totals	χ^2	df	*P*
Sex										
Male	18	21	33	31.4	6	4.6	57	2.69	2	>0.2
Female	19	15.5	21	23	2	3.4	42			
Total	37		54		8		99			
Maturity										
Mature	8	7.4	10	11	2	1.6	20	0.145	2	>0.9
Immature	29	29	44	43.5	6	6.3	79			
Total	37		54		8		99			

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NOTES

Large Floating Structure for Holding Adult Pacific Salmon (*Oncorhynchus* spp.)

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HUNTER, C. J., AND W. E. FARR. 1970. Large floating structure for holding adult Pacific salmon (*Oncorhynchus* spp.). J. Fish. Res. Bd. Canada 27: 947–950.

A large floating enclosure was used in a behavioral study to hold adult Pacific salmon in open sea water. The space enclosed approached the conditions of the natural environment and provided an excellent study area, almost completely free from the injuries and disease associated with the high water temperatures and abrasions caused by confinement of adult salmon in concrete ponds.

Received September 15, 1969

A FACILITY has long been needed for holding fish in salt water during experiments on their behavior, maturation, and survival. In the past, we tried to hold adult Pacific salmon in concrete saltwater ponds (70 × 20 × 3 ft); many fish soon died, were injured, or became diseased. As these conditions were apparently caused by high water temperatures and abrasion of fish against the concrete, we constructed a floating structure to hold experimental fish in open sea water.

This structure was used by biologists of the U.S. Bureau of Commercial Fisheries to study the escape of Pacific salmon from gillnets and the effects of gillnets on the salmon that escaped. The study was made at Reservation Bay, near Anacortes, Washington, from June 24 to October 17, 1968. Our work showed the value of the floating structure for holding adult salmon; in this note we describe the structure and report on its effectiveness.

The floating structure (Fig. 1) measured 162 × 41 ft; it provided two separate areas, each fitted with a net enclosure 10 ft deep — a large area for the experiments and a smaller one for holding the experimental fish after the tests. The net enclosures were of 15-thread 3-inch stretched mesh nylon. The structure provided a stable working platform with strength to resist storms and wave action.

The platform was made of units (Fig. 2) measuring 4 × 8 × 2 ft. Each unit consisted of polystyrene covered by plywood with exterior framing. The top frame members supported a walkway. The units were fastened together in sections by continuous ½-inch steel cables with a wood spacer (4 × 4 inch)

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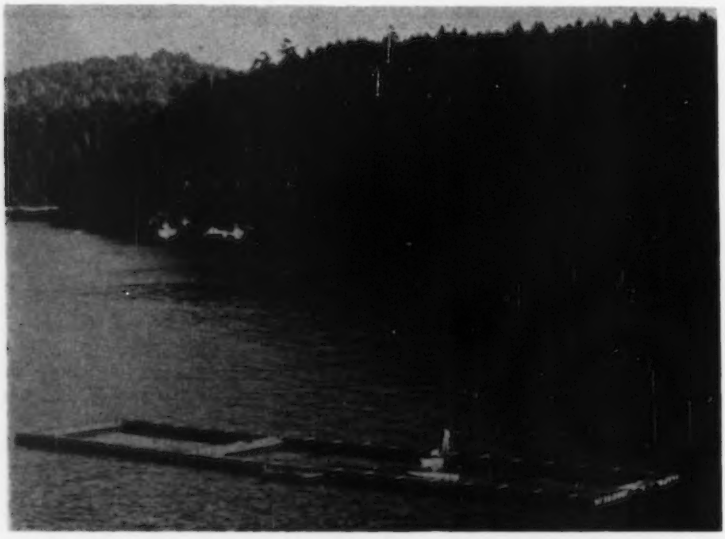


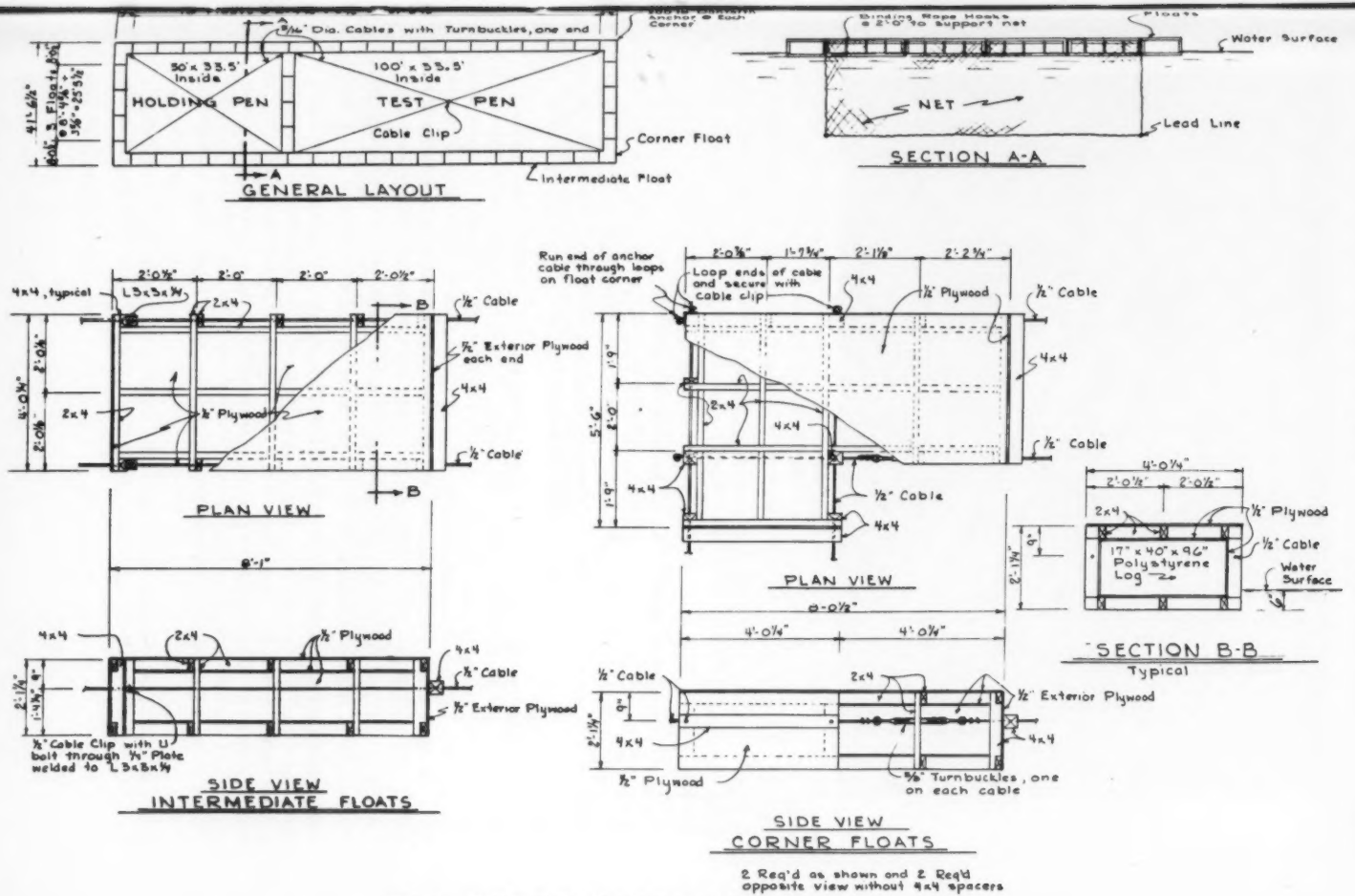
FIG. 1. Floating structure at test site, Reservation Bay, Washington, 1968. (Note large test area and smaller holding area.)

between each unit (Fig. 2). When tightened by turnbuckles, the cables held the platform rigid laterally but flexible vertically. The structural units were assembled into sections (Fig. 3) on the beach at low tide. At high tide the sections were floated off the beach, cabled together, and anchored in place. The structure was held in position by a 200-lb anchor at each corner. Each anchor cable had a depth–length ratio of 1:5. The structure was anchored in a small, shallow bay that was exposed to intermittent strong westerly winds but that had some protection from waves by an intervening reef. Depth at the site ranged from 13 to 26 ft because of tidal change, but stress on the platform caused by tidal currents was negligible.

Flotation of the structure was excellent; only 6 inches of the floats were submerged. Experiments were carried out safely during daylight in severe weather (up to 3-ft waves) but, for the safety of personnel, operations were suspended during darkness when waves reached a height of about $2\frac{1}{2}$ ft.

Red algae (*Antithamnion nigricans*), which grew on the netting, reduced abrasion between fish and the net, but created little restriction in passage of water through the net. The passage of fresh sea water through the netting kept the temperature of the water within the enclosure essentially equal to that of the bay.

The value of this structure for holding experimental fish is indicated by the survival of two groups of adult sockeye salmon (Fig. 4). These two groups of fish were captured under similar conditions and longevities would be supposed to be similar. One group, held in concrete ponds during 1967, had a survival



NOTES

FIG. 2. Details of floating structure including cross section of net.

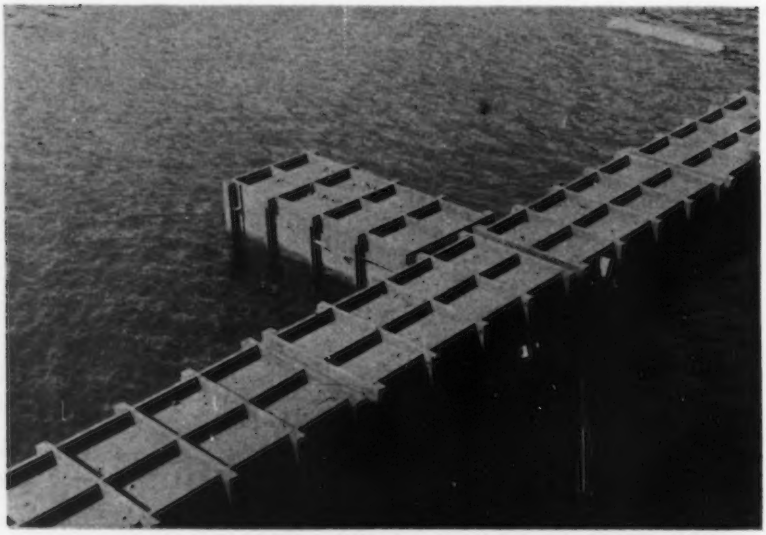


FIG. 3. Individual units cabled together in sections before final assembly of floating structure.

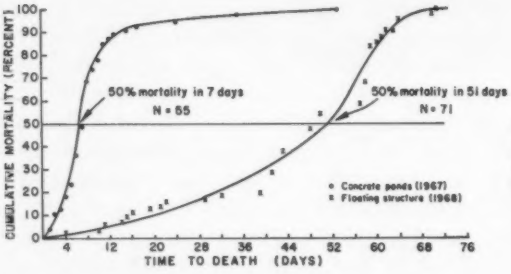


FIG. 4. Mortalities of adult sockeye salmon held in concrete ponds supplied with salt water and in a floating enclosure anchored in salt water.

rate of 50% after 7 days. The other group, held in the floating structure in 1968, had a survival rate of 50% after 51 days. The floating structure therefore provided the best survival conditions. Most fish that died in the floating structure after 51 days were sexually ripe and ready to spawn; some of these deaths may have been due to advanced maturity.

Plasma Protein Variation in a Winter Flounder (*Pseudopleuronectes americanus*) Population[1]

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PESCH, G. 1970. Plasma protein variation in a winter flounder (*Pseudopleuronectes americanus*) population. J. Fish. Res. Bd. Canada 27: 951–954.

The plasma protein variations of winter flounder (*Pseudopleuronectes americanus*) were studied by cellulose acetate electrophoresis. Eighty-eight fish caught in a single haul showed pattern differences associated with gonad maturation, sex, disease, and age. The groups of slow and medium mobility were prominent. A fast fraction with greater mobility than human albumin was present. Total concentrations increased with gonad maturation; in males the fast fraction was responsible, in females the slow fraction. Disease caused a drop in concentration for all fractions. Aging had associated concentration increases in the slow and medium fractions with no increase in the fast fraction.

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PLASMA PROTEIN ELECTROPHORETIC PROFILES are thought to be reliable indicators of physiological well-being. Variations in these proteins are intimately related to changes in the body's metabolic requirements, defense against invasion and injury, maintenance of body *p*H and osmotic pressure, and regulation of cellular activity and function (Booke, MS, 1962). The purpose of this note is to describe plasma protein variations found in a sample of winter flounder, *Pseudopleuronectes americanus* (Walbaum), taken in Narragansett Bay, R.I. The physiological states of the fish associated with particular protein profiles are reported. Because seasonal variations were not determined, the findings should be considered with reservation.

Materials and methods — Flounder were caught by otter trawl in the West Passage of Narragansett Bay in December 1964, 32 mature fish and 56 immature. The sample included two immature male flounder infected with "tail-rot." The infections resembled those described by Oppenheimer (1958) for Norwegian cod. In fish in advanced stages of the disease, Oppenheimer found motile rods in the blood suggesting that the infection was systemic.

The fish were kept in running sea water and bled within 2 hr. The caudal peduncle was severed, and the blood was collected in plastic tubes containing heparin (2 mg/ml of blood). After centrifugation at 10,000 rpm for 10 min, the samples were refrigerated until analysis, usually within 24 hr.

Electrophoretic analysis was carried out in a Shandon tank, with a modified Owens Buffer (Consolidated Laboratories, Inc., Chicago Heights, Chicago, Ill.) containing 5.0 g soluble barbitone, 3.25 g sodium acetate (hydrated), 34.2 ml 0.1 N hydrochloric acid, 0.34 g calcium lactate, and distilled water to 1 liter, *p*H 8.6. One microliter of the plasma was applied with a micropipette to cellulose acetate strips (18 × 2.5 cm; Serometrics, Inc., Chicago Heights, Chicago, Ill.) at 6 cm from the cathode. Four strips at a time were run with 5 mamp current, with a Heathkit power supply,

[1]Based on a thesis submitted in partial fulfillment of the requirements for the degree of Master of Science from the University of Rhode Island, Kingston, R.I., USA.

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for 1 hr at a temperature of 2 C. The strips were dried for 5 min at 100 C, stained for 15 min in 0.2% Ponceau S, rinsed twice in 5% acetic acid, and pressed between paper towels until dry. The strips were scanned with a Photovolt densitometer (models 501A and 52C), which recorded and integrated light absorbance. White light was used in this procedure.

Protein concentrations were estimated from the densitometry records with a human serum standard as a reference (Hyland Laboratories, Los Angeles, Calif.). Dye uptake in the protein fractions is independent of time and temperature, and Beer's law is obeyed (Scherr, 1961). Electrophoretic measurement of protein concentration is accurate according to Brackenridge (1960).

The proteins separated into three major groups, the division points being at approximately 2 and 4 cm from the origin (Fig. 1). For comparisons, the patterns were divided at these points on the electropherograms and labelled slow, medium, and fast fractions. Student's *t*-tests and correlation analyses (Snedecor, 1956) were used to determine the statistical significance of pattern differences.

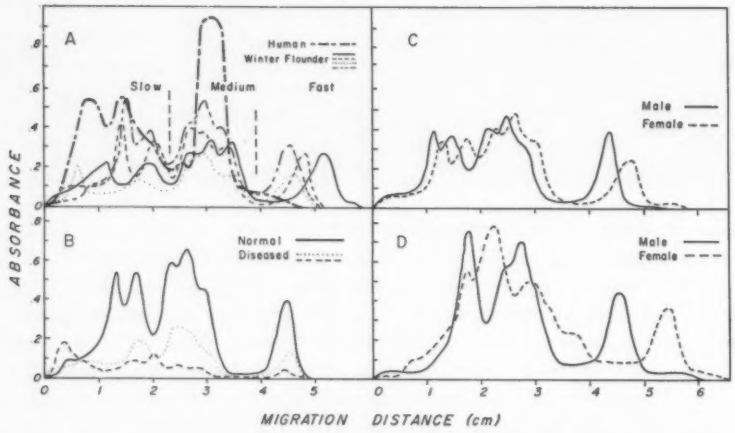

FIG. 1. Densitometry records of electrophoretic patterns for plasma proteins of *Pseudopleuronectes americanus* taken in December haul: (A) four randomly chosen individuals shown with human serum standard; (B) normal and diseased individuals; (C) immature individuals; (D) mature individuals.

Results and discussion — Plasma protein concentrations in the flounder were generally 3–4 g/100 ml of plasma. The slow and medium mobility groups were the more prominent fractions for all specimens sampled (Fig. 1). These findings agree with those of Sulya et al. (1960, 1961) who studied 26 species of fish from the Gulf of Mexico. A fast fraction with greater mobility than human albumin was present (Fig. 1A). Pattern differences were associated with gonad maturation, sex, disease, and age.

Within both sexes, the concentrations of the individual fractions tended to differ according to the stage of gonad maturation (Fig. 1C, D; Table 1) and the total concentrations were greater ($P = 0.05$) in mature than in immature fish (Table 2). In females, the slow fraction was responsible for the increase; in males, the fast fraction (Fig. 1C, D).

The great accumulation of protein in the sexual products prior to spawning is mediated by mobilization of proteins from the liver via the blood system.

TABLE 1. Mean curve areas of the various plasma protein fractions for *Pseudopleuronectes americanus* caught in a single haul in December 1964. The numbers represent integration units on a logarithmic scale.

		Fraction			
	No.	Slow	Medium	Fast	Total
Immature males	13	78.8±16.3[a]	101.9±30.9	29.7±11.0	210.4±44.5
Mature males	14	88.5±20.4	115.7±21.1	45.1±14.0	249.3±48.0
Immature females	43	68.4±23.7	87.9±25.9	30.6±10.4	186.8±53.7
Mature females	18	122.9±29.5	107.2±34.7	42.7±17.0	272.8±71.4

[a]One standard deviation of the mean.

TABLE 2. Statistical comparisons of mean curve areas of the various plasma protein fractions for *P. americanus* given in Table 1. The numbers are Student's *t* values. Each asterisk (*) represents a difference at the 95% confidence level.

	Immature female	Immature male	Mature male
Slow fraction			
Mature female	7.58*	4.85*	3.70*
Mature male	2.83*	1.36	
Immature male	1.47		
Medium fraction			
Mature female	2.39*	0.437	0.80
Mature male	3.63*	1.36	
Immature male	1.63		
Fast fraction			
Mature female	3.41*	2.40*	0.43
Mature male	4.15*	3.15*	
Immature male	0.27		
Total area			
Mature female	5.16*	2.71*	1.05
Mature male	3.85*	2.16*	
Immature male	3.56*		

Increased plasma protein concentrations are due to these proteins (Dessauer and Fox, 1959). The greatest change in the plasma protein patterns of ovulating females is due to the addition of vitellin, a phosphoprotein and lipoprotein complex that forms the yolk protein (Dessauer and Fox, 1959). Vitellin migrates as a globulin. The increased protein concentration of the mature males could be due to the transport properties of the fast fraction, or this protein could be serving as a source of amino acids for the concomitant protein buildup in the gonads.

The two immature males infected with tail-rot had very low total plasma protein concentrations, about one-half the normal levels (Fig. 1B.) Sano (1960) found similar decreases in diseased trout. The results may be explained

by the lack of plasma cells in teleosts (Engle et al., 1958). Without plasma cells, the liver is the main source of plasma proteins. Thus, liver damage, which may result from a systemic infection, would drastically reduce blood protein concentrations. Another possible explanation for these low concentrations is an osmotic imbalance caused at the infection site and leading to blood dilution.

Aging was associated with increased plasma protein concentrations. A correlation coefficient of 0.39 between total protein concentration and fork length was found for the 56 immature flounder in the sample. The protein fractions of slow and medium mobility were responsible. No increase was detected in the fast fraction. This pattern was reported for trout (Sano, 1960) and is thought to be due to increases in globulin antibody production as the animals are exposed to various diseases (Booke, MS, 1962).

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Occurrence of Whirling Disease of Trout in Western United States

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Whirling disease of trout, caused by *Myxosoma cerebralis*, was diagnosed in 1966 for the first time in rainbow trout (*Salmo gairdneri*) from California and Nevada hatcheries. In 1969 the first incidence of this disease in cutthroat trout (*S. clarki*) was reported in Nevada. To date these have been the only confirmed cases of whirling disease in western United States.

Received January 20, 1970

THE HIGHLY INFECTIOUS WHIRLING DISEASE of trout caused by *Myxosoma cerebralis* has been prevalent in hatcheries in the eastern United States since 1956 (Hoffman et al., 1962). This disease, however, was not diagnosed in any of the western hatcheries until 1966 when specimens sent to the Western Fish Disease Laboratory were confirmed histologically as having *M. cerebralis* infection. This report documents the first occurrence of the disease in the western United States.

In January of 1966 we received moribund, 9-month-old rainbow trout (*Salmo gairdneri*) from a private trout hatchery located on Garapata Creek just south of Carmel, California. In an accompanying letter the hatchery owner described the behavior as "an extremely nervous twisting action as though the fish were hooked in the mouth and then got off the hook. They spiral around and around as though they were tied by the nose. This becomes so violent at times that some of them come out onto the bank. This action seems to take place mainly when they are startled or when you approach the pond to feed." These signs were first noticed in a few fish when they were approximately 6 months old. At that time the hatchery owner thought that the problem was caused by either overcrowding or rising water temperature. However, the whirling behavior persisted and increased through the summer and winter months.

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Histopathological examination of these fish revealed an extensive myxosporidian infection in the cranial cartilaginous tissue (Fig. 1), particularly in the auditory capsule and base of the gill areas. Fresh wet mount preparations of the spores (about 6–10 μ in diameter) exposed to Lugol's iodine solution indicated the absence of iodinophilous vacuoles; this plus the cartilaginous location verified the identification.

In June of 1966 a group of 4½-month-old rainbow trout suspected of having whirling disease was sent to Western Fish Disease Laboratory from a state hatchery on the Truckee River in Nevada. Signs described by the biologist were those of typical *M. cerebralis* infection: tail chasing, whirling or twisting, and black tail. Histopathological examination revealed many trophozoitelike cells along the cartilaginous tissues of the cranial area, but no spores were observed. Subsequent examination of 7-month-old fish from the same lot disclosed the spores.

In late November 1969, a third case of whirling disease was reported from a population of rainbow and cutthroat (*S. clarki*) trout at a National Fish Hatchery in Nevada (W. J. Walsdorf, personal communication). Water supply was from wells and the Carson River. This is believed to be the first incidence of *M. cerebralis* reported in cutthroat trout.

Diagnosis of the California and the Nevada materials was confirmed by Dr Glenn Hoffman, parasitologist at the Eastern Fish Disease Laboratory.

The 1966 Nevada fish were planted in several water sheds before a definitive diagnosis was made. When *M. cerebralis* was definitely identified, the concrete troughs, tanks, and ponds were treated with live steam and thoroughly dried. No evidence of a recurrence has been found in the 3 years since the facilities were treated. The source of infection at this Nevada hatchery is unknown; however, it has a history of receiving wild and domestic strains of trout regularly from many sources in the western United States.

The approximately 100,000 rainbow trout from the Garapata Creek Hatchery in California were all destroyed by burial in compliance with California law. Again the source of infection is unknown. The hatchery, however, had a long history of receiving only trout eggs that are not believed to carry the disease (Hoffman et al., 1962). The water supply, however, contains a population of steelhead (*S. gairdneri*), and although infected specimens were not found at the time of the outbreak (12 fish were examined) they still remain as a suspected source of the infection.

To date the three cases noted above have been the only confirmed cases of *M. cerebralis* epizootics reported in the western United States.

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(FIG. 1 *opposite*)

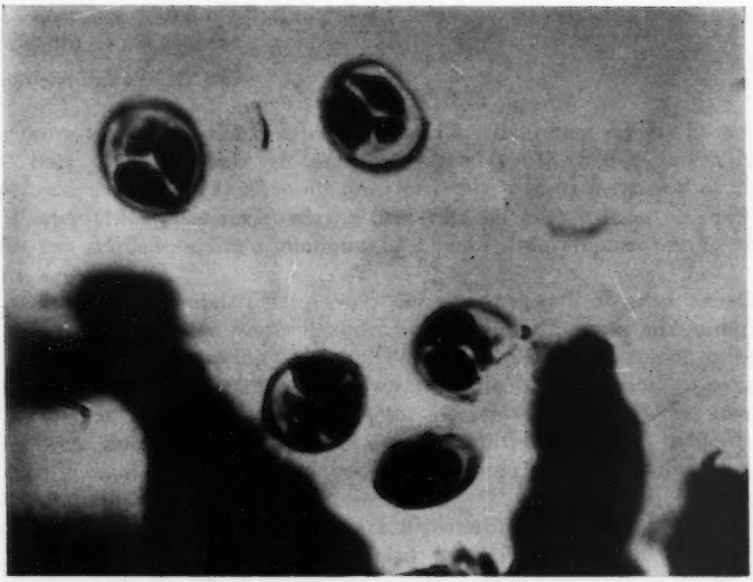

FIG. 1. *Myxosoma cerebralis* spores in the cranial area of rainbow trout. May-Grunwald-Giemsa stain; ×2060.

Further Evidence for a Three-Year Oocyte Maturation Time in the Winter Flounder (*Pseudopleuronectes americanus*)

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DUNN, R. S. 1970. Further evidence for a three-year oocyte maturation time in the winter flounder (*Pseudopleuronectes americanus*). J. Fish. Res. Bd. Canada 27: 957–960.

Evidence is presented for autumn growth of yolkless oocytes, which would tend to split the oocytes into two size groups.

Received November 27, 1969

DATA ON OOGENESIS in fish ovaries have been accumulating rapidly in recent years. Barr (1963), Braekevelt and McMillan (1967), Smith and Young (1966), and Wiebe (1968) have all described this process in teleost ovaries, and Blaxter (1969) has reviewed the literature on oocyte growth. Oocytes of most fish studied require a minimum of 2 years of development to become mature eggs.

Recently, Dunn and Tyler (1969) described aspects of gross anatomy and microanatomy of the winter flounder ovary. Winter flounder spawn during a short period in the spring. Bimodal distribution of diameters of yolkless oocytes in resting ovaries from experimental fish led these authors to suggest a 2- or 3-year maturation period for the oocytes. However, these resting ovaries were apparently in a special static condition probably induced by the experimental conditions, and oocytes exhibited no growth over the 4-month autumn sampling period.

In this study I examined the size distributions of yolkless oocytes in maturing ovaries in fish freshly sampled from Passamaquoddy Bay over a similar time period, with a view to relating any growth patterns to hypotheses on length of oocyte maturation period.

Winter flounder 35–45 cm in total length were obtained by otter trawl in Passamaquoddy Bay, N.B. Data were collected from 16 fish, four taken at each of four samplings (32-day intervals beginning on September 2, 1966). All fish had maturing ovaries. The left ovary of each fish was fixed in 5% formalin and later sectioned at 7 μ and stained with Gomori's trichrome. Details were given by Dunn and Tyler (1969). Oocyte diameters were sampled by measuring, with an ocular grid at 450 $\times$, the first 100 immature oocytes encountered and placing each in a 10-μ size class. Precision of measurement was

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limited by distortion of the cells by surrounding maturing oocytes. Distortion was greater in larger immature (yolkless) oocytes, and increased markedly with later sampling dates. Immature follicles in fish taken after December were extremely difficult to measure. Frequency distribution of the oocyte size classes for all fish are given in Table 1.

TABLE 1. Frequency distributions of diameters of immature oocytes in ovaries of 16 winter flounders. The symbol < indicates that the mean number of oocytes decreased with date; > indicates that the mean number increased.

		Size class (μ)												
Sampling date	Fish	1–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100	101–110	111–120	121–130
Sept. 2	1	1	5	9	12	14	10	11	13	5	6	5	7	2
	2	5	7	10	12	11	11	15	10	8	3	5	1	2
	3	0	10	6	13	18	17	15	8	4	3	4	2	0
	4	1	5	11	17	13	11	16	10	8	3	2	2	1
	Total	7	27	36	54	56	49	57	41	25	15	16	12	5
	Mean	1.7	6.7	9.0	13.6	14.0	12.3	14.2	10.2	6.3	3.8	4.0	3.0	1.3
Oct. 4	1	2	11	12	13	15	15	9	8	7	2	3	2	1
	2	0	4	9	10	6	14	20	13	10	5	6	3	0
	3	0	3	5	7	9	13	21	14	11	8	5	1	3
	4	2	5	6	14	14	12	17	11	11	3	2	2	1
	Total	4	23	32	44	44	54	67	46	39	18	16	8	5
	Mean	1.0	5.8	8.0	11.0	11.0	13.3	16.8	11.5	9.8	4.5	4.0	2.0	1.3
Nov. 5	1	1	3	10	15	15	7	15	16	9	4	2	3	0
	2	0	2	5	14	18	11	11	12	13	6	4	3	1
	3	2	6	8	10	9	13	15	9	14	5	3	3	3
	4	2	7	8	8	10	7	9	7	18	14	8	2	0
	Total	5	18	31	47	52	38	50	44	54	29	17	11	4
	Mean	1.3	4.5	7.8	11.8	13.0	9.5	12.3	11.0	13.5	7.3	4.8	2.8	1.0
Dec. 7	1	1	2	5	10	15	15	15	8	13	9	4	3	0
	2	2	1	10	9	12	14	18	11	11	10	2	0	0
	3	1	4	6	12	9	15	15	11	13	8	4	2	0
	4	3	4	6	9	8	12	7	12	14	12	10	2	1
	Total	7	11	27	40	44	56	55	42	51	39	20	7	1
	Mean	1.8	2.8	6.8	10.0	11.0	14.0	13.8	10.5	12.8	9.8	5.0	1.8	0.3
			<	<							>	>		<

Frequencies for each class were tested with the chi-square statistic on the null hypothesis that samples taken on different dates were all drawn from the same population. Only the two size classes shaded in Fig. 1 (80–100 μ) showed significant differences ($P = 0.05$) among dates, although five classes showed trends with progressively fewer (<) or more (>) eggs with time, as indicated in Table 1. Size classes 11–20 μ, 21–30 μ, and 121–130 μ had decreasing trends; classes 91–100 μ and 101–110 μ had increasing trends.

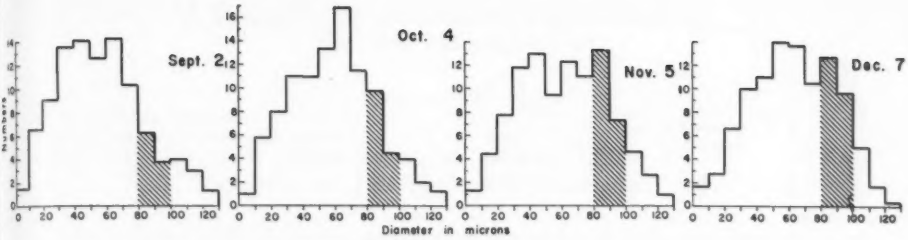

FIG. 1. Frequency distributions for diameters of yolkless oocytes for maturing ovaries in autumn. Shaded size classes had significant growth with time.

Since the proportion of oocytes in the smallest size classes remained low it is probable that few oocytes were added to the pool of immature follicles during the 4-month sampling period. Therefore, an increase in the proportion of eggs in larger size classes represented growth of a portion of the oocytes. That is, the additional eggs in size classes 80–100 μ came from smaller size classes. In a fixed number of follicles this process must have reduced numbers of smaller eggs, a fact borne out by the trend to fewer eggs in the size classes 11–20 μ and 21–30 μ.

Mean diameters (microns) and standard deviations (SD) for all oocytes measured on each date are presented below.

Sampling date:	Sept. 2	Oct. 4	Nov. 5	Dec. 7
Mean ± SD	56.7±25.2	59.8±26.1	61.9±26.9	62.2±26.0

Analysis of variance indicates no significant differences between means ($P >> 0.05$), although the increases with time suggest growth. Neither means nor standard deviations exhibit the marked increase with time that would be predicted on the basis of increased numbers of larger oocytes in the population. Examination of data in Table 1 helps to explain this discrepancy. Numbers of oocytes in the classes 111–120 μ and 121–130 μ, which greatly influence the mean and standard deviation, actually decrease with time. The decrease in numbers of oocytes in the larger classes could be due to atresia of the large immature oocytes which did not begin vitellogenesis.

It is unfortunate that methods used for cell measurement in this investigation did not allow reliable data to be collected through later stages of ripening ovaries. The increasing oocyte asymmetry is complicated by the fact that individual fish vary in their state of development at any given time. Nonetheless, the data did demonstrate that at least a segment of the oocyte population grew during the sampling period. If this process were continued, there could be a trend toward bimodality. It is my interpretation that two modes would become increasingly separated until a distribution was obtained similar to that presented for resting ovaries by Dunn and Tyler (1969) (Fig. 2). Yolkless oocytes in the larger size group (mode = 100–110 μ) were termed "large immature oocytes" and those in the smaller size group (mode =

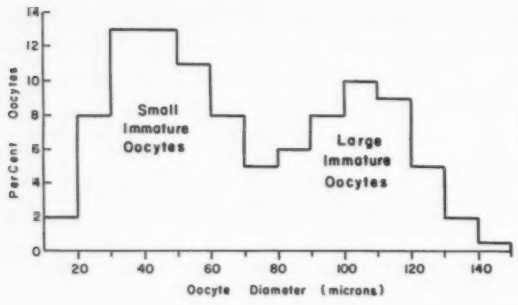

FIG. 2. Frequency distribution of oocyte diameters from resting stage ovaries. (From Dunn and Tyler, 1969.)

30–50 μ) were termed "small immature oocytes." The two stages of oocytes had morphological distinctions. Thus, the classes of oocytes that show numerical increase in the present data are tentatively identified with those oocytes that, by the following summer, would form the stock of large immature follicles, most of which begin vitellogenesis, to be spawned the next spring (Dunn and Tyler, 1969). If this interpretation is correct, then these data support the hypothesis for a 3-year oocyte maturation time in winter flounder.

One particularly intriguing question remains unanswered. This concerns the recruitment of small immature oocytes from the germinal epithelium. Is this a continual or a cyclic process? If cyclic, when does this process occur? In view of the cyclic development of large immature and maturing follicles, it would be surprising to find that this process is continual. In this study, oocytes in the smallest size classes formed a small proportion of the oocyte pool, and there was no significant difference in the numbers of these oocytes with time, indicating that recruitment is low from September to January. Barr (1963) described a similar situation for these months in plaice (*Pleuronectes platessa*). His more complete data showed that recruitment of oogonia was definitely cyclic in plaice, occurring in March and April, immediately after spawning.

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A Possible Optimization Experiment in Fishery Management

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A tag–recapture method for deciding whether a change in effort governing regulations will improve a fishery or the opposite is proposed. When the criterion is weight of catch, this reduces to seeing whether the total weight of fish recaptured in a certain tag–recapture experiment exceeds the total weight of fish released. Most of the many practical objections besetting this experiment are common to any method depending on tag–recapture methods to estimate rate of exploitation.

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FISHERIES SCIENCE is among those cursed in that all too often information is scavenged from nature rather than elicited through the stronger medium of controlled experiment. Indeed, controlled experiments are unusual enough to be an interesting novelty, and the one described herein is presented partly as such a novelty and partly as a conceptual device illuminating mark–recapture methods in fisheries assessment.

Optimization methods commonly rest on the device of observing the result of a small perturbation of the system; this small change may be made experimentally or by calculating what would happen if it were made. The standard calculus method of setting the derivative to zero uses the device of the small perturbation; so also, to cite an example from fisheries science, does the proof of Allen's (1953) lemma.

In applying this idea to optimizing some aspect of fishery regulation, say the number of vessels, we might perturb the situation by adding one more vessel, a research ship, and seeing if this makes things better or worse. The actual measure of "betterness" might be total weight caught, total value caught, or profit to the fishermen, etc. But there is an obvious flaw; after adding the experimental ship we know the result of the perturbed fishery, but what would have been the result had the extra vessel been withheld? The experiment lacks a control. Obviously having the control demands the operating of two fisheries at the same time and this is impossible. The control however, can be simulated by a marking experiment: the catch of the experimental vessel is marked and released. (To avoid trivial complications, the problem of those fish in unfit condition to release is postponed.) Some of the released fish are later recaptured by the fishery. If the total weight, value, etc., of the fish recaptured exceeds the total weight, etc. of the fish released, then the perturbation was for the worse and the optimum number of vessels is fewer, not more. If not recorded, size of recapture can be estimated from

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size at release, time of recapture, and known growth parameters. We have simulated the noncatching of fish by the extra vessel by releasing those it catches.

The experiment is a standard mark–recapture experiment. The only novelty, such as it is, is the interpretation of it. More usually, the experiment would be used to measure the ratio of fishing to natural mortality, the exploitation rate; this would be used as a parameter in some population dynamics model from which an assessment would be derived. The main point of this note is that the population dynamics model, with its attendant assumptions, complications, and calculations, is superfluous; the experiment furnishes an assessment without it.

The same principle applies to other fishery regulations. To test whether a closed season should be shortened or lengthened, a small amount of experimental fishing could be done in the closed season and the catch marked and released. The experiment does not apply to closed seasons associated with reproduction of the stock; here the benefit or lack of it will show up in later generations rather than in this one.

Mesh size regulations are a bit more complicated. Changing the whole industry to a slightly different mesh size is impractical. However, we can send out an experimental ship with a smaller mesh. This changes the number of vessel-days as well as the mesh size; our control should be the same experimental ship fishing with standard mesh. Thus, the difference between the experimental treatment and control treatment is only those fish caught by the small mesh that would have slipped through the larger one, and these are the only fish marked and released. The small change in vessel-days is the same for both experimental treatment and control. The reader may have noticed the close relation between this argument and Allen's (1953) lemma.

Fish that are in too poor condition to release are included in both the experimental and control fishing and can be ignored.

It is basic that the difference between the experimental and control fishing must be, in a small way, the difference between the imposition and nonimposition of the regulation in question. This is easy to say; it may be hard to accomplish in practice, as indicated below: if the regulation applies to a considerable area, can the experimental vessel afford to cover all of it? If the fishery has a seasonal variation, can the experimental vessel sample the entire season? Both these questions indicate that the experimental vessel's effort must be governed by some experimental design that may require randomization; the details of such a design may be complicated and troublesome. If the fish unfit to release are (say) the small ones, additional fish must be withheld to bring the size distribution of released fish back into line; conceivably the same thing could arise with sex as well as age distribution. In the mesh size experiment, it would be extremely difficult to identify fish caught by the small mesh that would have slipped through the larger one. It would rarely be possible to do this at all accurately by the simple process of subtracting one proportional size distribution from another, since changes

in mesh size often affect catching efficiency at the upper as well as at the lower end of the size distribution.

The more obvious experimental errors — extra mortality of tagged fish, inhibited growth of tagged fish, nonreturns, tag loss, etc. — decrease the weight, value, etc. of reported recaptures. Thus the experiment is conservative: if it shows fishing is too heavy, it understates the overfishing. The conclusion that fishing is too light may call for reserving judgment. It hardly need be said that fisheries more usually need regulating downwards.

The problems and objections of the last two paragraphs are formidable, and the practical fishery manager will often, perhaps always, reject this experiment on such grounds. But it must be remembered that the same objections apply to most methods, however elaborate and sophisticated, that rely on tag–recapture methods to estimate the exploitation rate. Indeed, the value of this experiment may lie, not in its application, but in its conceptual simplicity. The innumerable practical objections may be less evident, though equally cogent, to a more elaborate assessment couched in the framework of a mathematical population dynamics model.

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Use of Ultraviolet Light to find Parasitic Nematodes in situ

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PIPPY, J. H. C. 1970. Use of ultraviolet light to find parasitic nematodes in situ. J. Fish. Res. Bd. Canada 27: 963–965.

The parasitic nematodes *Anisakis* sp., *Contracaecum aduncum*, and *Phocanema decipiens* fluoresce when irradiated by ultraviolet light (360 mμ). Fluorescence greatly simplifies searching for the nematodes in situ when they are not deeply embedded in host tissues. Also, differences in fluorescence quality enable immediate preliminary identification of specimens. The method is particularly well suited for long-term investigations or when accurate counts are necessary.

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MANY LIVING AND DEAD ANIMAL TISSUES show visible fluorescence when irradiated with ultraviolet light (Encyclopedia Britannica). Ortolani and Campanile (1966) used this characteristic in general epidemiological surveys

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for vaginal trichomoniasis and found that examination of fresh material with a fluorescence microscope was superior to standard microscope inspection after staining. Sinnhuber and Law (1949) first reported the use of ultraviolet light to help find parasitic nematodes in fish muscles. Other workers studying bacteria (Pulvertaft, 1934; Slavnina, 1948; Wasserman, 1965) and lichens (Cernohorsky, 1950, 1959) have demonstrated that the colour of fluorescence may be dependent on the species or strain of organism involved. In this paper the use of autofluorescence to help find and identify parasitic nematodes in situ is discussed.

Method and materials — Fluorescence characteristics of the following parasitic nematodes were determined: larval and adult *Contracaecum aduncum* and larval *Anisakis* sp. from Atlantic salmon (*Salmo salar*); adult *Anisakis* sp. and *Phocanema decipiens* from grey seals (*Halichoerus grypus*); larval *P. decipiens* from Atlantic cod (*Gadus morhua*); and adult *Philonema agubernaculum* from Arctic char (*Salvelinus alpinus*). Except for larval *Anisakis*, which were examined before and after freezing, all examinations were made with previously frozen material. In situ observations were made on external surfaces of salmon viscera for larval *Anisakis* and *C. aduncum*, and on the inner surfaces of seal stomachs for adult *P. decipiens*. These were made with a 15-w ultraviolet light of 360 mμ (Fisher Multi-Ray Lamp No. 11-988VI with No. 11-988-4 long wave light). Specimens were viewed through ultraviolet protective goggles (Fisher No. 11-403) to eliminate reflected ultraviolet and near ultraviolet blue light. Photographs were made using an amber filter (Nikon A12) and Kodak Panatomic –X film.

Examinations were carried out in the lighted laboratory or in the field by the use of a reasonably light-proof chamber (Fig. 1) with the ultraviolet light source mounted inside. Specimens to be examined were placed inside through a door on the right side and the examiner's hands and arms were inserted into the arm holes underneath the light-proof curtain.

Results and discussion — All species tested, except *P. agubernaculum*, fluoresced. Live *Anisakis* had a very pale fluorescence but specimens frozen before examination fluoresced brilliantly. A similar change in fluorescence of nematodes after death was observed by Ronald (1956) who suggested that coagulation of protein may be involved. The change is also similar to Manohar's (1969) finding that the intensity of fluorescence of fish muscle after freezing is always greater than that before freezing. The following table illustrates the fluorescence characteristics of the nematodes examined:

larval and adult *Anisakis* — brilliant bluish-white
larval and adult *C. aduncum* — pale to bright yellow
larval *P. decipiens* — brilliant bluish-white
adult *P. decipiens* — variable: pale to bright yellow to pale to bright bluish-white possible in different areas of the same specimen.

When two species with different fluorescence characteristics were found in a single sample of hosts (*Anisakis* sp. and *C. aduncum* only were present in the body cavity of the salmon examined), immediate preliminary identification and sorting of the specimens was possible. All the nematodes fluoresced much brighter and with a different colour than the surrounding tissues (seal stomachs, orange; salmon viscera, red to pale yellow). Thus, the nematodes were readily visible under ultraviolet light even when only a fraction of a millimeter of

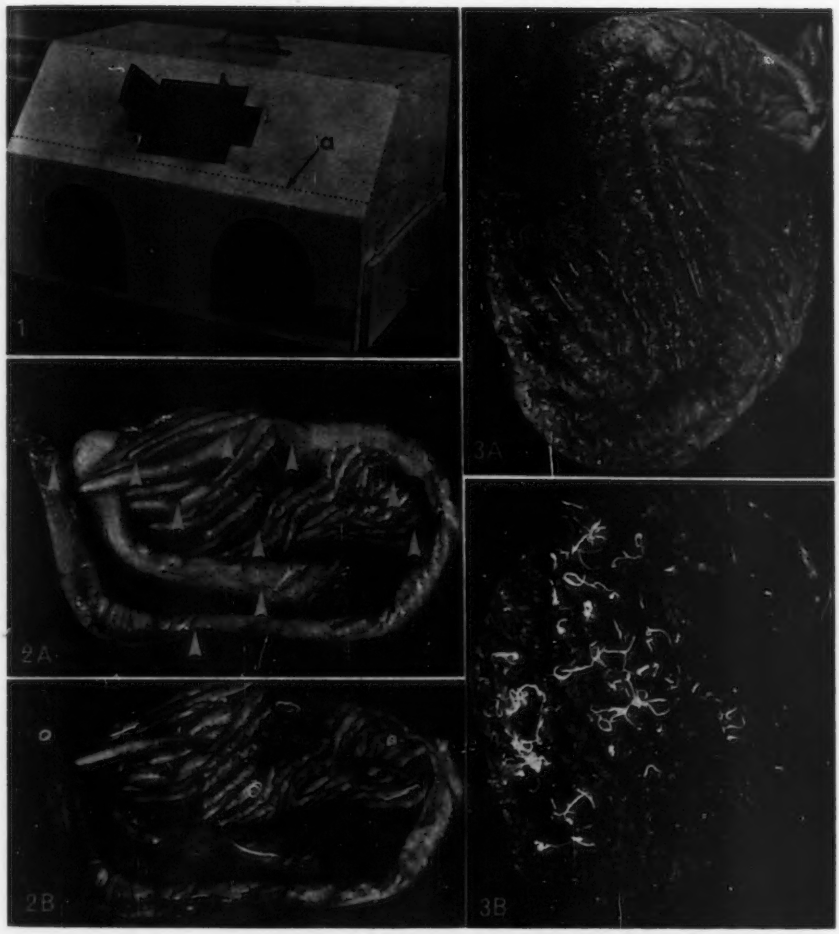

FIG. 1. Apparatus used to find autofluorescent nematodes in normally lighted laboratory or in the field. (*a*) Location of attachment of light-proof curtain. Curtain is draped to 8 inches below bottom of chamber.

FIG. 2. Alimentary canal of an Atlantic salmon with 10 *Anisakis* sp. (*pointers*) as observed by (A) reflected light, and (B) autofluorescence.

FIG. 3. Opened seal stomach containing adult *Phocanema decipiens* and *Anisakis* sp. observed by (A) reflected visible light, and by (B) autofluorescence caused by ultraviolet light.

Pippy — J. Fish. Res. Bd. Canada

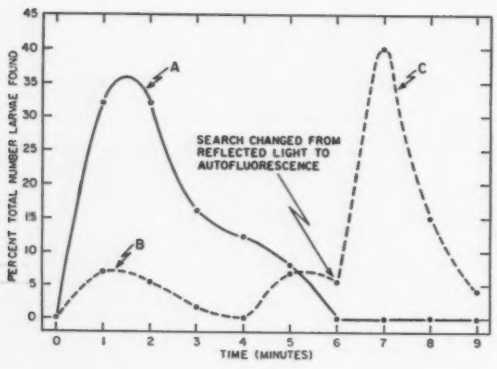

FIG. 4. Relative efficiency of finding *Anisakis* larvae on the viscera of Atlantic salmon by searching with: (A) autofluorescence caused by ultraviolet light (total 25 larvae found), and (B) reflected visible light. Curve C shows additional larvae found when viscera used in (B) were examined under ultraviolet light for an additional 3 min (total 57 worms for curve B–C).

their body length was in view. Because ultraviolet light does not penetrate deeply in animal tissues, particularly the dense tissues of the gut wall, and the intensity of light emitted by the worms is low, nematodes embedded more than about 0.5 mm below the tissue surface were not visible with the light intensity used here. This approach is therefore particularly well suited for finding nematodes encysted in or on the visceral and parietal petitoneum or free in the abdominal cavity (Fig. 2), as well as for locating nematodes attached to the stomach or intestinal mucosa (Fig. 3) and partially hidden among the rugae. Ultraviolet examination provides an efficient means for checking internal surfaces of alimentary canals after unattached nematodes have been removed using conventional methods (washing, settling, and decanting). The use of much more intense ultraviolet sources is recommended for finding nematodes embedded deep in fish muscles (Sinnhuber and Law, 1949).

Since some parasitic nematodes, such as larval *Anisakis* and *C. aduncum*, are small and almost invisible against the background of hosts' tissues (Fig. 2A and 3A) accurate counts are difficult and time-consuming. In an experiment involving examination of the external surfaces of the viscera from two samples of 11 salmon each, the same examiner found 26% (maximum estimate) of the worms present in the first 6 min of searching with visible light (Fig. 4, curve BC). Much higher efficiency was noted when ultraviolet light was used during the first 6 min (Fig. 4, curve A). Similarly high efficiency was noted by Sinnhuber and Law (1949) who used ultraviolet light to find parasites in muscle tissues. The use of fluorescence to find parasitic nematodes seems particularly well suited for long-term investigations or where accurate counts are necessary.

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Habitat Characteristics of the Pacific Hagfish, *Polistotrema stouti*

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Environmental information from Mayne Bay on Barkley Sound, Vancouver Island, indicates that the Pacific hagfish, *Polistotrema stouti* (Lockington), occurs on substrates consisting mainly of silt and in water characterized by high near-bottom salinities (31–32‰) but low surface salinities (24‰) into which they occasionally swim. Near-bottom dissolved oxygen concentrations were low, an observation in harmony with the high oxygen affinity of hagfish hemoglobin.

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Hagfish habitat features are generally believed to include a soft mud or clay substrate and high-salinity low-temperature water. The available information is based on surprisingly few field observations, supplemented with information from physiological studies (Bigelow and Schroeder, 1948; McFarland and Munz, 1958; Adam and Strahan, 1963; Foss, 1963).

The environmental information described below was obtained on October 21, 1968, from Mayne Bay on Barkley Sound, Vancouver Island, when more than 200 Pacific hagfish, *Polistotrema stouti*, were caught for later experimental work.

The traps used were similar to those described by Adam and Strahan (1963). Water quality information was obtained using standard oceanographic techniques and analyses (Strickland and Parsons, 1968). Bottom samples were taken with a Kahlisco mud snapper dredge and analysed according to techniques described by Folk (1965).

It is assumed that hagfish are permanently resident in this locality since they have been observed in the same area on two successive years, and short fishing periods (as little as 20 min) were productive.

Salinity, temperature, and dissolved oxygen profiles are shown in Table 1 for two stations (Fig. 1). The sediment analyses are shown in Table 2.

The observations are interesting in several respects. This is the shallowest recorded occurrence (20 fath) of this species in B.C. waters. According to Clemens and Wilby (1961), the maximum recorded depth of this hagfish is 455 fath indicating an overall range that parallels that of *Myxine glutinosa* 15–524 fath (Bigelow and Schroeder, 1948).

Although near-bottom salinities are high (31–32‰) there is a steep halocline between 5 and 10 m with surface salinities of about 24‰. Night-lighting observations showed that this species sometimes swam near the

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TABLE 1. Salinity, temperature, and dissolved oxygen depth profiles, Mayne Bay on Barkley Sound, Vancouver Island. Refer to Fig. 1 for station locations.

Station no.	Sample depth (*m*)	Salinity (‰)	Dissolved oxygen (*ml/liter*)	Temp (*C*)
1	Surface	23.89	5.79	10.1
	5	24.18	5.78	10.3
	10	30.76	4.71	10.6
	20	31.24	4.03	10.5
	30	31.12	3.35	10.6
2	Surface	24.19	5.78	10.1
	5	25.78	5.66	10.2
	10	29.74	5.04	10.4
	20	31.54	3.26	10.3
	30	31.63	2.77	10.4

TABLE 2. Bottom sample analysis, Mayne Bay on Barkley Sound, Vancouver Island. Refer to Fig. 1 for station locations.

Particles[a]		% composition by weight	
Type	Size (*mm diam*)	Sample 1	Sample 2
Medium and coarse sand	≥0.25[b]	0.5	0.3
Fine sand	≥0.125	0.5	0.4
Very fine sand	>0.0625	0.5	0.7
Silt[c]	<0.0625	74.5	74.6
Clay	≤0.0039	24.0	24.0

[a]Classified according to the Wentworth scale (Folk, 1965).
[b]Mainly shell fragments and organic debris.
[c]Silt and clay determined by hydrometry.

surface, so that for short periods they were exposed to reduced salinities. Their ability to tolerate lower salinity levels than *M. glutinosa* (Adam and Strahan, 1963) is consistent with the above observations.

The observed temperature (10 C) may be near the upper limit for this species. For *M. glutinosa* the upper limit as indicated by geographic distribution is around 10–13 C (Bigelow and Schroeder, 1948).

Perhaps the most interesting observation concerns dissolved oxygen. Manwell (1958) observed that the hemoglobin of this species has an affinity for oxygen "as high if not higher than any known vertebrate blood." The environmental oxygen profile indicates concentrations of around 3 ml/liter (4.3 mg/liter) at 30 m. Probably near-bottom concentrations would be even lower. Waldichuk (1956) recorded concentrations as low as 1.5 mg/liter in other regions of the sound, equivalent to a partial pressure of approximately 25 mm Hg. According to Manwell's (1958) oxygen equilibrium curves, this is an adequate loading tension for hagfish blood so that their hemoglobin

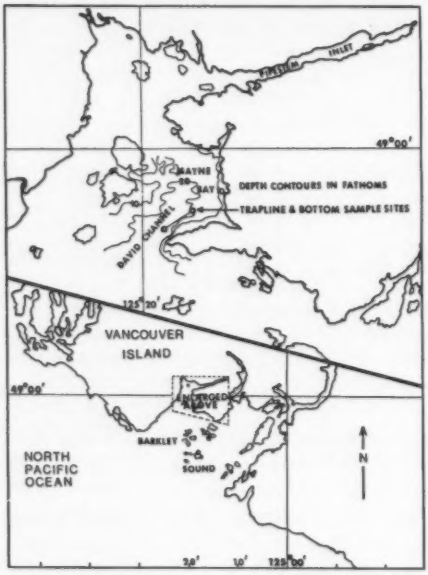

FIG. 1. Barkley Sound, Vancouver Island, showing hagfish and environmental data collection sites (open circles). Station 1 is the more northerly collection site. Information from Canadian Hydrographic Service charts 3001 and 3627.

is well adapted to providing an oxygen transport function in the environmental situation described.

Finally, the results of the sediment analysis for the first time provide a quantitative characterization of the bottom type utilized by this species indicating a high proportion of material <0.0625 mm in diameter (clay).

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Prevalence of Naturally Acquired Agglutinating Antibodies Against *Aeromonas salmonicida* in Hatchery Trout in Central Pennsylvania

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KRANTZ, G. E., AND C. E. HEIST. 1970. Prevalence of naturally acquired agglutinating antibodies against *Aeromonas salmonicida* in hatchery trout in central Pennsylvania. J. Fish. Res. Bd. Canada 27: 969–973.

A survey for naturally acquired antibodies against *Aeromonas salmonicida* in brook and brown trout in central Pennsylvania hatcheries showed great variation in the percentage of reactive individuals (0.8–87%) within 24 populations. Antibodies were not detected in fish less than 12 months old, and the percentage of serologically reactive individuals increased with the age of the population. Prevalence of antibodies in yearling trout populations in which furunculosis was epizootic was about twice as great as in fish experiencing enzootic furunculosis.

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DURING EXPERIMENTAL INVESTIGATIONS of the immune response of brook trout, *Salvelinus fontinalis*, and brown trout, *Salmo trutta*, fish with naturally acquired agglutinating antibodies against *Aeromonas salmonicida* were found in hatchery populations that had no record of clinical furunculosis (Reddecliff, MS, 1960; Krantz et al., 1964a, b). Obviously these populations contained fish that had experienced subclinical furunculosis or were carriers of the disease. Therefore, a serological survey of 24 trout populations ranging in age from 6 to 40 months and known to have experienced furunculosis of widely different severity was undertaken to describe the epizootiology of furunculosis in hatchery-reared trout in central Pennsylvania.

Background knowledge of the pattern of development and retention of agglutinating antibodies against *A. salmonicida* in brook and brown trout (Krantz et al., 1964a, b) validates the use and interpretation of the serological information. In experimental studies, trout injected with bacteria (viable or formalin-killed without adjuvant) produced titers (1:20–1:320) similar to those acquired naturally (1:2–1:160). Trout injected with viable bacteria

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developed maximum antibody titers (1:20–1:2560) 3 months after injection. Titers were observed to decline to values ranging from 1:2 to 1:160 approximately 7 months after injection, and these antibody levels persisted for at least a year. This pattern of immune response in brook and brown trout was consistent with recent descriptions of the immune response of fishes (Post, 1966; Ridgway et al., 1966; Summerfelt, 1966; Hodgins et al., 1967).

The survey was conducted from the spring of 1961 to the fall of 1963 by sampling 24 different populations of brook and brown trout from state hatcheries in central Pennsylvania ("Bellefonte" strain of brook trout, Snieszko et al., 1959; Wolf, 1954). Fish that comprised a population were selected randomly from a single raceway that contained trout of a known age. Benner Spring hatchery, which has nonconfluent raceways, was considered to contain several separate populations. Water temperatures of the hatcheries ranged from 10 to 18 C.

The serological samples, taken by cardiac puncture, were collected and tested by a rapid slide test as described previously (Krantz et al., 1964a). Titer is expressed as the reciprocal of the highest serum dilution showing macroscopic agglutination, and includes the dilution by the test antigen. Because of dilution in the slide test technique, a positive test using undiluted plasma is reported as a titer of 2 (a 1:2 dilution of plasma in test antigen and diluent). Doubtful or partially agglutinated reactions occurred with undiluted plasma, but are not included in the results. The range and mode of titers in the population provided a better representation of the agglutinin frequency than did the mean, which was influenced by individual fish with an extreme titer.

A wide range of variation was observed in the prevalence of agglutinins against *A. salmonicida* in the populations of both brown trout (0–87% positive) and brook trout (0–60%) (Table 1). The diversity in prevalence of agglutinins within the 24 trout populations indicates a continuous, widespread interaction

TABLE 1. Prevalence (ranges) of naturally acquired agglutinins against *Aeromonas salmonicida* in populations of brook and brown trout.

Age (*months*)	Brown trout					Brook trout				
	Total no. fish	No. populations	% positive	Agglutinin titers		Total no. fish	No. populations	% positive	Agglutinin titers	
				Modes	Range				Modes	Range
6	70	2	0	–	–	70	1	0	–	–
10	81	2	0	–	–	52	1	0	–	–
12	–	–	–	–	–	55	1	0	–	–
16	708	7	0–32	5–20	2–160	567	7	0–8	10–40	5–80
20	10	1	50	20	2–40	9	1	4	10	2–20
22	625	6	8–87	5–40	2–320	537	7	4–60	10–40	2–320
26	–	–	–	–	–	61	1	13	10	5–320
30	92	2	44–57	2–80	2–320	85	2	12–24	10	2–80
36	19	2	40–64	10	2–320	14	1	7	2	–2
40	110	2	20–47	10–20	2–320	–	–	–	–	–

between individuals of the host population and *A. salmonicida*. Such factors as time of infection, percent infected, survival rate, and production of detectable antibodies are some of the variables that may have contributed to the great amount of variation in antibody prevalence. The range in percentage of fish with detectable antibodies in these populations was similar to the range of mortality (0–97.8%) among 18 brook and brown trout populations in New York studied by Wolf (1954). The level of contact with *A. salmonicida* under hatchery conditions was almost as high as mortality resulting from experimentally induced infections (Snieszko et al., 1959). The serological data indicate that many populations of hatchery trout in central Pennsylvania have higher levels of contact with *A. salmonicida* than has been indicated by studies of "carrier" fish. McGraw (1952) reviewed the findings of several investigators who isolated viable *A. salmonicida* from 3 to 34% of the apparently healthy trout they sampled. Rabb and McDermott (1962) reported 0–38.2% "carrier" brook trout in an Ontario stream population.

Agglutinins were not detected in fingerling trout less than a year old (Table 1). Agglutinins may not have been found because fingerling trout rarely recover from infection by *A. salmonicida*. This hypothesis would be consistent with the high natural mortality that accompanies epizootics in young trout. However, fingerling trout survive and respond immunologically to parenteral injections of viable *A. salmonicida* under experimental conditions (Krantz et al., 1964a). Blake and Clark (1931) found brown trout fry less susceptible to experimental infection than older trout, and suggested a high level of resistance in young fish. Failure to detect agglutinins in trout less than a year old lends support to their hypothesis. It is also pertinent that the LD_{50} dose per animal for fingerling trout was almost as great as for yearling trout (Krantz et al., 1964a).

In general the percentage of serologically reactive trout increased as the age of the populations increased (Table 1). This suggests an accumulation of individuals that had experienced disease, especially since the modes and ranges of titers of reactive sera differed little among populations of various ages. Brook trout populations generally had a lower prevalence of detectable agglutinins than brown trout populations of the same age. This phenomenon was observed in brook and brown trout reared in the same hatchery (Benner Spring), where the water source and rearing procedures were essentially the same for both species. The difference in prevalence of agglutinins may be influenced by an observed difference between the two species in the rate of decline of agglutinin titers produced by parenteral injection of adjuvant vaccine (G. E. Krantz, unpublished data). Brook trout began to show a decline in titer about 12 months after immunization, whereas titers in brown trout remained high for over 24 months.

Fourteen populations of yearling trout were sampled in the spring (16-month-old fish) and again in the fall (22-month-old fish) to document the effects of epizootic and enzootic furunculosis on the prevalence, mode, and range of agglutinin titers in yearling trout. During the intervening months

daily mortalities were recorded by hatchery personnel, and bacteriological examinations of morbid fish kidneys were conducted weekly to determine if *A. salmonicida* was present. Trout populations that experienced enzootic furunculosis had very low mortality, and *A. salmonicida* was isolated only rarely from the few morbid fish. These populations exhibited satisfactory growth and were considered healthy by fish culturists. In contrast, populations in which *A. salmonicida* was epizootic experienced high losses of fish for short periods during the summer. Morbid trout had lesions characteristic of furunculosis from which *A. salmonicida* was isolated. Sulfonamides and tetracyclines were administered to these populations to suppress the disease.

Those populations that experienced epizootic furunculosis during the summer showed a greater increase in serologically reactive fish than did populations that experienced enzootic furunculosis (Table 2). The modes (5–40)

TABLE 2. Percentages of yearling brook and brown trout with detectable agglutinin titers in spring and fall in populations with enzootic or epizootic furunculosis. Numbers of fish tested in parentheses.

Enzootic furunculosis			Epizootic furunculosis		
Population	Spring	Fall	Population	Spring	Fall
Brook trout					
Pleasant Gap	0.8(130)	6.9(189)	Benner Spring-2	0(42)	14.3(35)
Paradise	0(151)	4.6(172)	Benner Spring-3	0.8(36)	24.0(25)
Jersey	0(38)	13.3(45)	Benner Spring-4	0(42)	9.6(41)
Benner Spring-1	0.8(128)	8.2(61)	Benner Spring-5	1.2(83)	24.2(33)
Brown trout					
Paradise	10.9(137)	24.9(201)	Pleasant Gap	2.3(125)	30.5(105)
Benner Spring-6	12.3(163)	17.5(137)	Benner Spring-8	10.3(29)	58.0(43)
Benner Spring-7	7.5(161)	29.5(112)	Benner Spring-9	27.2(44)	65.0(40)

and range (2–160) of titers in the populations experiencing enzootic furunculosis were very similar to the modes and range in populations that experienced epizootic furunculosis. These results indicate a similarity in the severity of infection among survivors of epizootic and enzootic furunculosis. Mortality data were not adequate to determine the relative levels of severity of the disease among the populations.

The value of serological techniques to define the past disease experience of trout populations is demonstrated. The acquired knowledge should be of considerable significance in studies of experimental immunization, disease resistance, and fish survival in selective breeding programs.

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Tissue Carotenoids in Prespawning and Spawning Sockeye Salmon (*Oncorhynchus nerka*)

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CROZIER, G. F. 1970. Tissue carotenoids in prespawning and spawning sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd. Canada 27: 973–975.

Spawning sockeye salmon from the Wauk Wash River, B.C., contained 65% less carotenoid than prespawning fish taken at sea near Juneau, Alaska. Free astaxanthin in the muscle of spawning fish was only 1% of the amount noted for prespawning fish. Ninety-five percent of the pigment retained by the spawning males was located in the skin as esters and 85% of the pigment of spawning females was deposited in the eggs as free astaxanthin.

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THIS IS A REPORT on comparisons of samples of prespawning and spawning sockeye salmon (*Oncorhynchus nerka*) concerning carotenoid contents of the muscle, skin, and gonads. The familiar flesh coloration of sockeye salmon is due to the presence of free astaxanthin (Bailey, 1937), but the only reference to skin carotenoids in the species concerns an aberrant color phase (Evelyn, 1967). Carotenoid transport to skin and eggs has been well established in other salmonids (Goodwin, 1950; Steven, 1949; Peterson et al., 1966), but the system of the sockeye salmon has never been defined nor have tissue concentrations been reported previously.

Procedures — Prespawning fish were obtained in ocean waters near Juneau, Alaska, and frozen prior to analysis (2 weeks); spawning salmon were taken from the Wauk Wash River and flown to the laboratories, where analysis was completed within 3 days.

The muscle of all specimens and gonads of the females were ground in absolute ethanol and reextracted until the pigment was removed from the tissue. The skin (dorsolateral) was treated in like manner but a solvent of ethanol/hexane (1/1) was substituted. The pigments were then washed into hexane and the spectral curve taken with a Beckman DB recording spectrophotometer. From the optical density thus obtained the concentration of carotenoid in the crude extract was approximated using an extinction coefficient of 1840 at 470 nm for astaxanthin. From the weight of tissue extracted and the total calculated for the specimen, both the tissue carotenoid concentration and total carotenoid content were obtained.

Thin-layer chromatography (Brinkmann precoated plates of silica gel G and a solvent system of 15% acetone in hexane) was used for testing the homogeneity of the extracts. No critical effort was made to identify each of the xanthophyll esters from the skin.

Results and discussion — The prespawning fish, male and female, contained high concentrations of carotenoid in the muscle (Table 1), giving it the characteristic color. The muscle extract yielded only a single component with one broad absorption maximum at 470 nm. This profile is usually associated with astaxanthin (3,3′dihydroxy,4,4′diketo-β-carotene), the pigment

TABLE 1. Average concentrations of carotenoids in tissues of prespawning and spawning sockeye salmon according to sex, and percentages of total contents. Ranges in parentheses.

No. in sample	Sex	Skin			Muscle			Gonad			
		Concn (*mg/100 g*)	Total (*mg*)	% of total	Concn (*mg/100 g*)	Total (*mg*)	% of total	Concn (*mg/100 g*)	Total (*mg*)	% of total	Total (*mg*)
					Prespawning						
3	Male	0.29 (.12–.39)	0.31 (.09–.45)	2	2.80 (2.3–3.4)	35.30 (33.7–36.5)	98	–	–	–	35.6
2	Female	0.32 (.30–.34)	0.33 (.24–.41)	1	2.30 (1.3–3.2)	33.90 (19.4–48.4)	90	0.92 (.88–.96)	2.8 (2.6–3.0)	9	37.0
					Spawning						
3	Male	4.50 (1.0–9.5)	12.70 (2.7–25.7)	95	0.03 (.01–.04)	0.39 (.15–.68)	5	–	–	–	13.1
4	Female	1.10 (.49–1.9)	2.00 (.83–4.16)	14	0.03 (.003–.05)	0.26 (.04–.5)	1	2.95 (2.9–3.0)	9.8 (8.4–11.2)	85	12.1

known from salmon (Bailey, 1937). In the biphasic system (hexane/ethanol) the astaxanthin was hypophasic indicating that the pigment was in the unesterified form. Thin-layer chromatography supported this conclusion.

In the skin of the prespawning fish the concentrations of carotenoid were much less than in the muscle, but similar in the two sexes. The skin yielded three fractions of esterified xanthophylls: two esters of astaxanthin, which comprised the majority, and a minor chromatographic spot that was unidentified.

Though the spawning fish were almost certainly not from the same population as the prespawning fish, the visible reversal of the pigment depots at spawning is obvious. If the two stocks had approximately equal levels of premigratory lipids and lipid pigments, apparently more than 60% of the carotenoids were metabolized. But the portions retained by the two sexes were deposited in different areas, exclusively in the skin of the males and primarily in the eggs with less in the skin of the females. A functional significance for egg carotenoid has been suggested (Hartmann et al., 1947; Hubbs and Stavenhagen, 1958), but the mechanism has never been elucidated. The role of skin pigmentation as a breeding stimulus has been established by Tinbergen (1953), but the importance to the sockeye can only be inferred at this time.

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A Relation of Size to Swimming Speed in Rainbow Trout[1]

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Cruising speed at 10 C of rainbow trout 4–100 g was measured in a rotating chamber and found to vary as weight$^{0.12}$. Weight varied as length$^{2.91}$, so that swimming speed was dependent on length$^{0.4}$, close to the relation found for other salmonids by other authors. A 10-g fish could swim at approximately 9 body lengths (L) sec^{-1} whereas at 100 g the cruising speed was 5.5 L sec^{-1}. By extrapolation the rate at 1000 g was 3 L sec^{-1}.

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A BRIEF STUDY of the relation of size to swimming speed was undertaken with yearling rainbow trout, *Salmo gairdneri* Richardson, to obtain a correction factor to remove the effect of size differences in the relation of cruising speed to temperature.

Methods — Yearling rainbow trout, 6–20 cm fork length, were acclimated to 10 C and tested at the same temperature. The tests were performed between July 23 and September 17 during the normal working day. All fish were of the same parentage but drawn from three lots reared respectively at 5, 9, and 15 C until hatching. They were distinctively finclipped according to their rearing history and a record was kept of their origin when the swimming speed was determined.

Swimming speed was determined in an open rotating annular chamber (cf. Fry and Hart, 1948) with the greater diameter of the cavity being 50.5 cm and the lesser 40.9 cm. The depth of water was approximately 15 cm. As a reference point, a 60-w lamp was placed 30–50 cm from the chamber, the outer wall of which was transparent. An air stone, approximately 3 × 5 cm, was suspended in the chamber and served as a further reference point as well as to aerate the water. Cruising speed was recorded as the number of revolutions per minute. The slippage factor was determined to be 0.83 by observing the rate of revolution of balls of cotton wool suspended in the water as compared with the rate of rotation of the chamber.

The fish was placed in the chamber and the chamber rotated slowly. The fish was initially oriented into the counter-rotatory position by a tap on the head with a glass rod if pointed in

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the opposite direction, or on the tail if facing in the right direction but drifting with the rotating water. Such stimuli were given only as the fish passed the reference point. When the fish was swimming steadily, the rate of rotation was increased in steps of 5–8 rpm at intervals of approximately 1 min until approximately 30 rpm. At higher speeds the rate of rotation was then increased by some 2–5 rpm each step. At the rate where the fish lost place by one or more laps in a minute, the trial was continued for 3 or 4 min, then the rate of rotation was reduced to the last speed where the fish maintained its place. The rate of revolution was then measured by a 1-min count of revolutions during which the fish swam steadily. Each fish was weighed after test but not measured. A weight–length relation was derived from a second series of dead fish from the same lots.

Results and discussion — The observations are presented as a scatter diagram in Fig. 1. The regression of $\log_e$ speed (rpm) on $\log_e$ weight calculated from these data is $Y = 3.503 + 0.128\ (\pm 0.014)\ X$, $N = 128$. The similar regression of weight (g) on fork length (cm) was $Y = -3.965 + 2.908\ (\pm 0.033)\ X$, $N = 702$. There is a possibility that a higher rearing temperature was associated with a higher swimming speed but the effect, if any, was slight and all data were pooled.

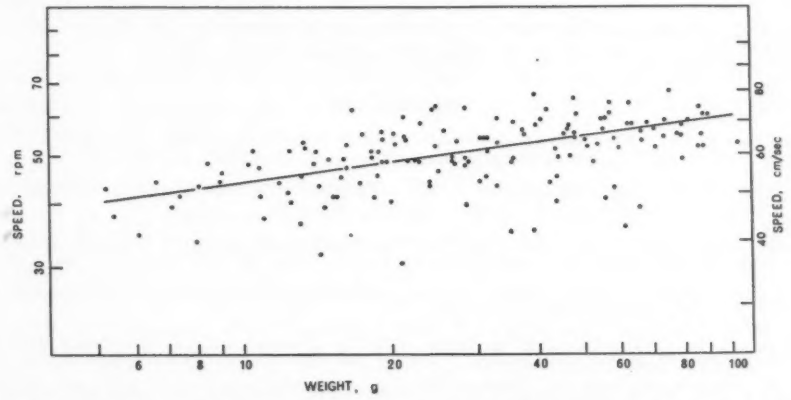

FIG. 1. Plot of original observations. To estimate speed in distance travelled, multiply by mean circumference of chamber and slippage factor, i.e. 143.6 × 0.83.

Earlier expressions of swimming speed in relation to size have been referred to length. Our data can be compared with these through the weight–length exponent. Thus Bainbridge (1960, 1962) found a speed–length exponent of 0.58 for the rainbow trout (*Salmo gairdneri*) and a weight–length exponent of 3.1. Hence his data yield a speed–weight exponent of 0.155, which is similar to the present finding. Blaxter and Dickson (1959) present a curve for the brown trout (*Salmo trutta*) that also shows a similar relation. Brett (1965) found the exponent for the speed–length relation in another salmonid, the sockeye salmon (*Oncorhynchus nerka*), to be 0.5. Weight–length exponents in fish rarely vary outside the limits of 2.9–3.1 so that we

can confidently expect that the speed–weight relation will have been 0.16–0.17 values, which are also not likely significantly different from the present value for the rainbow trout.

These various relations were obtained in various different types of apparatus. For example, Bainbridge used a closed rotating wheel in which the water was locked to the rotation by special vanes and presumably rotated as a body while Brett used a water tunnel in which fine turbulence was generated to maintain a constant cross-sectional flow. It seems likely, therefore that the cruising speed of the salmonids varies essentially as $L^{0.5}$ or $W^{0.16}$. Many other fish presumably behave similarly.

However, speed–length exponents of the order of unity have also been reported for fish. Blaxter and Dickson (1959) found the speed of herring, *Clupea harengus*, to be proportional to the body length as did Bainbridge (1960) in the dace, *Leuciscus leuciscus*. Pavlov et al. (1968) present similar data for *Perca flavescens* and three minnows. Houde (1969) also found an exponent of unity for young yellow perch (*Perca flavescens*) and the related walleye (*Stizostedion vitreum*) over lengths of 10–20 mm.

Thus it still seems, as Bainbridge (1962) believed, that there are major specific or familial differences in the relation of speed to size in fish but this belief should be tested by a thorough comparative study.

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Mortality of Dart-Tagged Captive Sablefish (*Anoplopoma fimbria*)

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Infection from dart tags killed sablefish (*Anoplopoma fimbria*) in a tank and in a net. Mortality began 3 months after tagging and reached a high level after 8 months that persisted for more than a year. At the observed mortality rate virtually all would die within a year.

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DART TAGS may cause heavy mortality for more than a year after tagging. Mortality from dart tags was noted during a growth study of sablefish, *Anoplopoma fimbria*.

Except that its plastic tubing was only about 4 cm long, the tag was the FT2 illustrated by Everhart and Rupp (1960). Tag and applicator were disinfected just before application and the tag was applied as described by Everhart and Rupp (1960).

On September 17, 1968, 32 sablefish, average weight 1.7 kg, were tagged. They had been held since February 20, 1968, without any mortality after March 14, in cylindrical Fibreglas tanks, 2.4 m in diameter, filled to about the 60-cm level with sea water pumped from Departure Bay at 4 gal per minute. Tagged fish were returned to the same tanks.

One fish died on December 15 and two on December 16, 1968. Autopsy indicated that each death resulted from infection attributable to the tag. A pocket of pus about 25 mm in diameter surrounded the barbed head of each tag, the tag's pointed inner end being near the deepest margin of the pocket. Tags had penetrated about 2 cm deeper than original insertion depth. Presumably, movement inward would be easy because of tissue breakdown from infection, whereas movement the other way would be prevented by the barb. A fourth fish died on December 19, 1968, and a fifth on January 14, 1969, both badly infected at the tag. On January 15, large swellings were noted on two fish near their tags and, when lanced, several cubic centimeters of fluid drained from each. Close examination showed lesser swellings on several others.

On January 15, 1969, all the tags were removed by pulling on the protruding ends. In most instances the tags were quite loose and they protruded less than when applied. Because of the barbs, sizeable holes resulted through which, in most instances, fluid escaped. On February 14, a large swelling near the tagging site on one fish was lanced and drained. Deaths of two fish (the

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sixth and seventh) on February 14 and March 1, respectively, were attributed to tag-induced infection. Four more fish died before the experiment was terminated on September 16, 1969, probably of causes unrelated to tagging.

Sablefish were also held in a retaining net in Departure Bay (Kennedy, MS, 1968) where they were untended except for regular feeding and periodic transfer to an identical fresh net when the occupied net became laden with algae, etc. Because the net bottom was about 5 m below the water surface, the net had to be "dried up" before live fish could be counted or dead ones recovered. Intervals of approximately 1 month between inspections resulted in deterioration of dead fish before recovery. Several either completely disintegrated or escaped.

Dart-tagged sablefish were put into the retaining net as follows: 3 on July 19, 21 on July 23, and 54 on October 16, 1968. By August 13, 1969, none of the 78 were left: 18 had been subsequently removed while in good condition for use elsewhere, 30 dead fish had been recovered, and the other 30 had presumably died and disintegrated between inspections. Possibly some of the latter escaped, but it is unlikely. Decomposition usually made the cause of death indeterminable but — except for two fish wounded by great blue herons — in no fish was death attributed to any cause other than tag-induced infection. The last fish to die had been tagged on July 23, 1968, and died August 13, 1969, with considerable infection around the tag.

Among the tagged fish there were roughly 0.6 deaths per fish-year between July 23, 1968, and April 24, 1969, and roughly 6 deaths per fish-year between April 24 and August 8. A "fish-year" is 1/365 of the sum of several products, each product being the average number of fish alive in the net multiplied by the number of days that there were that number of fish in the net. The same net had been used to hold untagged fish from April 25, 1968, to July 23, 1968, during which time there had been roughly 0.3 deaths per fish-year.

Thus, when dart tags were applied to a group of sablefish in a tank where none had died for 6 months, most of them showed signs of tag-induced infection in 3–4 months and 22% of them died from it — 16% in a single month. When the tags were removed, infection abated. The connection between tagging and mortality was not quite as direct for fish held in the net. However, the relation was evident from a mortality rate among tagged fish 20 times as great as among untagged fish under similar conditions. So, in both tank and net, infection from dart tags killed sablefish at a rate that would leave few, if any, within a year. It apparently was at least 3 months after tagging before any died, and at least 8 months before mortality reached a maximum. Mortality rate was high for at least a year, when all the tagged fish had died.

When fish in the tank were weighed or when those in the net were transferred periodically, they had to be lifted from the water by dipnet. Sablefish struggle violently in a dipnet and their struggles may have promoted infection from the tags by causing the barbs to work deeper into tissue that was softened by infection. Also, the shorter than usual plastic tubing on the tags may have contributed to infection; longer tubing would likely cause more drag, which

might tend to keep the tag from working deeper. Finally, dart tags might not affect other species as seriously; the shape of sablefish is such that tags must be inserted more nearly vertically than in some species, which could encourage infection. Despite these qualifications dart tags should be regarded as a potential source of heavy and prolonged mortality.

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Occella to Supersede *Occa* for a Genus of Agonid Fishes

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BAILEY, R. M., AND C. G. GRUCHY. 1970. *Occella* to supersede *Occa* for a genus of agonid fishes. J. Fish. Res. Bd. Canada 27: 981–983.

The generic name *Occa* Jordan and Evermann (1898), applied to several agonid fishes of the North Pacific Ocean, is preoccupied by *Occa* Chesnon, 1835 (Aves). *Chesnonia* Iredale and Whitley 1969 was proposed as a substitute for the junior homonym, but *Chesnonia* is a junior synonym of three generic names proposed by Jordan and Hubbs (Mem. Carnegie Mus. 10: 93–346, 1925). From these we select *Occella* (type species, *Agonus dodecaëdron* Tilesius) to replace *Occa* Jordan and Evermann. *Iburina* Jordan and Hubbs, a subjective generic synonym of *Occella*, is retained for a subgenus of *Occella*; *Iburiella* is treated as a subjective subgeneric synonym of *Iburina*.

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AS HAS BEEN DEMONSTRATED by Iredale and Whitley (1969), *Occa* Jordan and Evermann, 1898 (Agonidae, Pisces), is preoccupied by the overlooked *Occa* Chesnon, 1835 (Anatidae, Aves), erected for a single species, the eider duck, *Anas mollissima* Linnaeus, 1758. *Occa* Chesnon is currently regarded as a junior synonym of *Somateria* Leach, 1819. In light of the foregoing, Iredale

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and Whitley proposed a substitute name, *Chesnonia*, with *Brachyopsis verrucosus* Lockington as the type species, to replace *Occa* Jordan and Evermann, the junior homonym. In so doing, however, they overlooked three earlier and available names, all proposed by Jordan and Hubbs (1925, p. 290–291), that we (and others) interpret as generic synonyms of *Occa* Jordan and Evermann. These are *Occella* (misspelled *Ocella* in the key on p. 290), orthotype *Agonus dodecaëdron* Tilesius, 1810; *Iburina*, orthotype *Occa iburia* Jordan and Starks, 1904; and *Iburiella*, orthotype *Iburiella kasawae* Jordan and Hubbs, 1925. These three plus *Occa* were viewed by Jordan and Hubbs as a group of related monotypic genera, which they characterized in a key.

More recently most authors have been more chary in their recognition of genera. Iredale and Whitley (1969), indeed, included both *dodecaedron* (=*Occella*) and *iburia* (=*Iburina*) in *Chesnonia*. Freeman (1951; MS, 1951) classified *Occa* and *Iburina* as subgenera of *Occa* Jordan and Evermann. Andriashev (1954, p. 424, and 1964, p. 456) recognized *Occella* as a subgenus of *Occa*, a genus that he said included several species placed in the genera *Occa*, *Occella*, *Iburia* (lapsus for *Iburina*), and *Iburiella* by American authors. In a summary treatment Matsubara (1955, p. 1166) reduced the nominal genera of Jordan and Hubbs (1925) to subgenera of *Occa*. Gruchy (1970) agrees with Freeman's (1951; MS, 1951) assessment of the subgeneric division of *Occa* (i.e., two subgenera). The three names proposed by Jordan and Hubbs (1925) in the Agonidae — *Occella*, *Iburina*, and *Iburiella* — are interpreted by us as junior generic synonyms of *Occa* Jordan and Evermann, and, as such, any one of these is available to supersede it.

Acting under our prerogative as first revisers [International Code of Zoological Nomenclature, Article 24 (a)] we select *Occella* Jordan and Hubbs 1925, in preference to *Iburina* and *Iburiella*, as the generic name to replace *Occa*. *Occella* (gender feminine), a diminutive of *Occa*, is closest to that familiar name in orthography, it holds line precedence, and its type species (*dodecaedron*) is believed to be more closely related to *verrucosa* than to *iburia* or *kazawai*.[1] For these reasons *Occella* is selected as the generic name. The genus, which contains six species from the North Pacific Ocean, may be divided into two subgenera: *Occella* Jordan and Hubbs includes *O. dodecaedron* (Tilesius), *O. verrucosa* (Lockington), and an undescribed species. *Iburina* Jordan and Hubbs (with *Iburiella* Jordan and Hubbs as a synonym) contains *O. iburia* (Jordan and Starks), *O. kazawai* (Jordan and Hubbs), and *O. kuronumai* (Freeman). The subgenera and species will be discussed and characterized elsewhere by Gruchy (1970).

The synonymy of the genus *Occella* is as follows:

Occa Jordan and Evermann, 1898, p. 2043. Type species by original designation, *Brachyopsis verrucosus* Lockington. Name preoccupied by *Occa* Chesnon, 1835 (Aves), type species by monotypy *Anas mollissima* Linnaeus, 1758.

[1]We emend the spelling of the specific epithet *kasawae* to *kazawai* as Jordan and Hubbs (1925, p. 293) stated that the species was named for Mr Masunosuke Kazawa.

Occella Jordan and Hubbs, 1925, p. 291 (misspelled *Ocella* on p. 290). Type species by original designation, *Agonus dodecaëdron* Tilesius, 1813.

Iburina Jordan and Hubbs, 1925, p. 290–291. Type species by original designation, *Occa iburia* Jordan and Starks, 1904. Regarded as a subgenus of *Occella*.

Iburiella Jordan and Hubbs, 1925, p. 290–291. Type species by original designation, *Iburiella kasawae* Jordan and Hubbs, 1925.

Chesnonia Iredale and Whitley, 1969, p. 45. Type species by original designation, *Brachyopsis verrucosus* Lockington.

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Influence of Body Size on Silvering of Atlantic Salmon (*Salmo salar*) at Parr–Smolt Transformation

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JOHNSTON, C. E., AND J. G. EALES. 1970. Influence of body size on silvering of Atlantic salmon (*Salmo salar*) at parr–smolt transformation. J. Fish. Res. Bd. Canada 27: 983–987.

Large yearling Atlantic salmon parr turn silvery more quickly than small parr when held under a series of controlled temperature and photoperiod regimes. Photoperiod appeared to exert a negligible influence on silvering, and water temperature was the main controlling variable.

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JOHNSTON AND EALES (1968) recently showed that the increased dermal purine deposition in Atlantic salmon at parr–smolt transformation is highly dependent on water temperature and negligibly affected by daily photoperiod manipulation. However, these authors thought that the tendency to smoltify also depended on the size of the fish. Several workers suggested a relation between size and the onset of parr–smolt transformation in Atlantic salmon (Allen, 1944; Elson, 1957; Evropeitseva, 1957; Parry, 1958; Pinder and Eales, 1969) and in other salmonids (Houston, 1961; Conte et al., 1966; Vanstone and Markert, 1968). This study investigated the influence of body size on the development of silvering in smolts under the controlled temperature and photoperiod regimes used previously (Johnston and Eales, 1968).

Yearling parr were obtained in September 1967 from the Miramichi Fish Culture Station, South Esk, N.B. They were held until December 1967 in the laboratory in running dechlorinated Fredericton city water at seasonal temperatures and photoperiods in four large plywood holding tanks (Johnston and Eales, 1968). In early December, two nylon screens were placed in each tank to provide three equal compartments, and fish were separated by fork length into three groups: <10 cm, 10–12 cm, and >12 cm. On December 21, each of the four tanks containing three sizes of juvenile salmon was subjected to one of the four temperature and photoperiod regimes used in earlier work (Johnston and Eales, 1968; Pinder and Eales, 1969). The regimes were

Temp	Photoperiod	Code
3±1 C from Dec. until Mar. 12, then increasing to 14 C by June	Lengthening from 8 hr in Dec. to 15 hr in June	NLNT (natural light, natural temp)
	8 hr Dec. to June	8LNT (8 hr light, natural temp)
3±1 C from Dec. to June	Lengthening from 8 hr in Dec. to 15 hr in June	NL3T (natural light, 3 C)
	8 hr Dec. to June	8L3T (8 hr light, 3 C)

On alternate days fish in each tank were fed approximately 200 g ground beef liver, which had been gelatinized to prevent excess turbidity in the compartments.

Once each month the fork length, fresh weight, and the visually assessed state of silvering (parr, silvery parr, or smolt) were recorded for each fish using criteria previously described (Johnston and Eales, 1968). Each fish was then returned alive to its assigned compartment. Chemical determinations were not used because fish would have to be sacrificed making it impossible to follow silvering in the same fish from December to June. Furthermore, a good agreement between silvering and purine levels had been previously demonstrated (Johnston and Eales, 1967, 1968). The number of fish in each compartment and their state of silvering for each condition are summarized in Fig. 1. The size of each monthly sample varied because of a slight incidence of mortality and the removal of a number of fish for other experiments. Seasonal changes

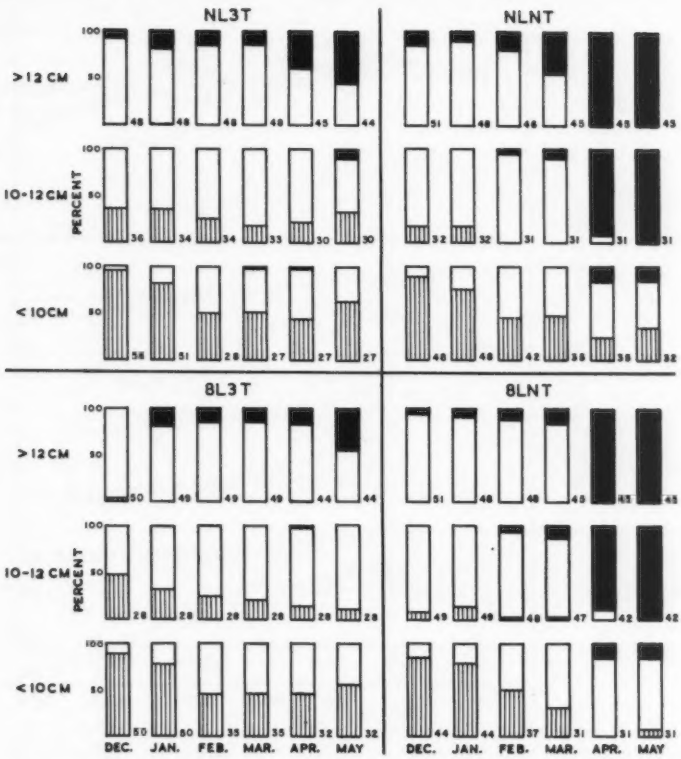

FIG. 1. Seasonal changes in the occurrence of parr ▥, silvery parr □, and smolt ■ in three populations of salmon (>12 cm, 10–12 cm, <10 cm in December) held under four different temperature and photoperiod regimes. The numbers refer to the number of fish visually assessed each month. The codes used are identified in the text.

in the coefficient of condition, and fresh weight and fork length are shown in Fig. 2 and 3, respectively.

These data show that, regardless of the size of fish considered, there was a pronounced influence of water temperature on silvering and the coefficient of condition. This was particularly evident after the mid-March rise of temperature in natural temperature groups. There did not appear to be any influence of photoperiod on silvering, although under natural temperature conditions the coefficient of condition in the larger fish (>12 cm and 10–12 cm) was slightly lower on natural photoperiod than on 8-hr photoperiod. All these data support the previous experiments by Johnston and Eales (1968).

These data also demonstrate that the larger the fish the more rapid is the development of the silvery appearance. In fact, in December, in all four

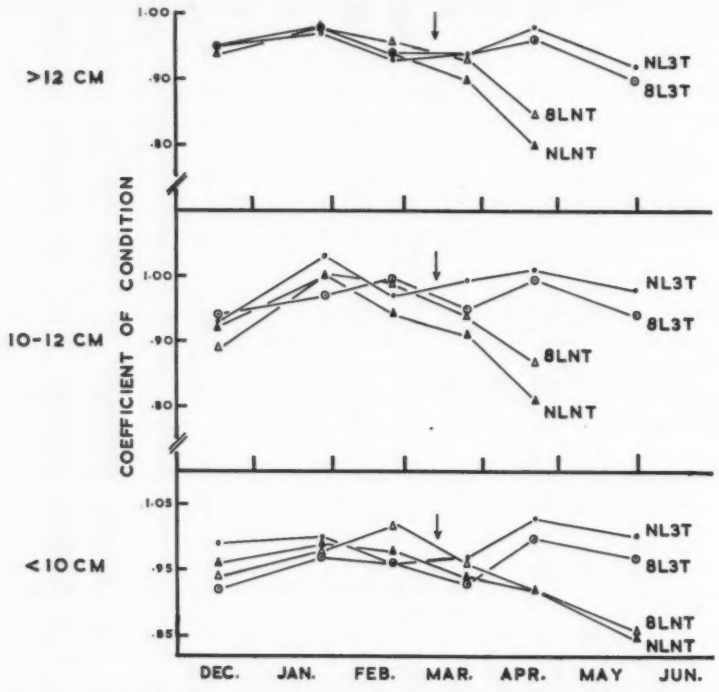

FIG. 2. Seasonal changes in the mean coefficient of the condition in three populations of salmon (>12 cm, 10–12 cm, <10 cm in December) held under four different temperature and photoperiod regimes. The arrow signifies the increase in temperature in NT regimes on March 12.

regimes the majority of fish >12 cm were silvery parr. Silvery parr characteristics had developed well in advance of any temperature rise or lengthening of photoperiod. At the other extreme were the fish <10 cm, which were almost entirely parr in December and of which only a small percentage eventually became smolts even under natural temperature regimes. In the fish <10 cm there was also a slight tendency for the percentage of parr to increase in May. This may represent the phenomenon of desmoltification that was described by Evropeitseva (1962).

In conclusion, although temperature is a key factor in regulating the extent of purine deposition during parr–smolt transformation, its effect is highly dependent on the size of fish considered. An influence of daily photoperiod on body silvering has yet to be demonstrated in the Atlantic salmon.

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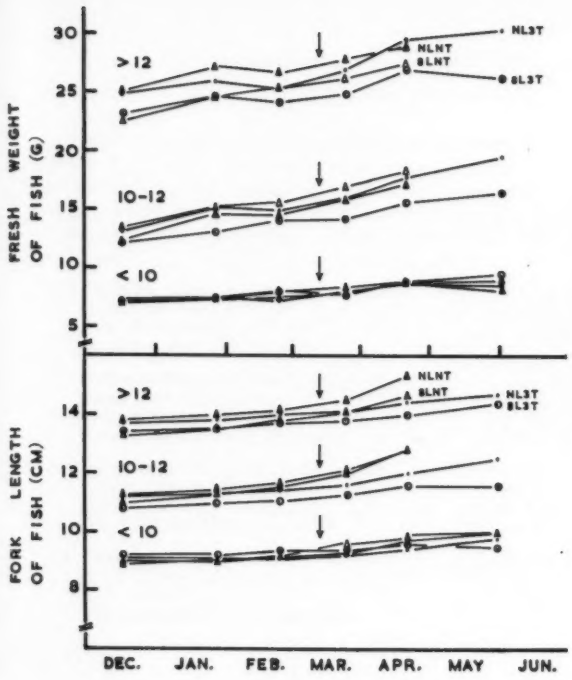

FIG. 3. Seasonal changes in the fresh weight and fork length for three populations of salmon (>12 cm, 10–12 cm, <10 cm in December) held under four different temperature and photoperiod regimes. Each point represents the mean value of each assessed group (see Fig. 1 for group numbers). The arrow signifies the increase in temperature in NT regimes on March 12.

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